# NUTRITIONAL CONSTRAINTS ON EGG PRODUCTION IN THE BLUE TIT

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## Abstract

Food supply is known to be a major factor influencing timing and level of investment in avian reproduction and the nature of its influence has been a subject of research for many years. Most previous studies, however, have considered food primarily in terms of energy supply. In this research I have investigated the possibility that other nutrients, particularly protein, may proximately constrain egg production. An experimental approach, involving the provisioning of breeding blue tits with supplementary foods of differing nutritional composition, was combined with observation and analysis of the birds' natural foraging sites and prey availability around the time of egg formation.

Early in the breeding season (15-29$^{th}$ April), before most birds had begun to lay, approx. 76% of all observed foraging occurred in oak, with the remainder observed predominantly in birch. By mid to late May, however, when the majority of birds had begun laying, blue tits were observed to forage almost exclusively in oak (~98% of foraging records). Over the whole observation period around 92% of all arboreal foraging was recorded on twigs, buds and leaves.

Systematic sampling of arthropods at the identified foraging locations of breeding blue tits was carried out concurrently in order to examine prey availability. In oak, arthropods were found to be relatively scarce during mid to late April, increasing in abundance through the laying period of the population. The predominant arthropods present were Lepidopteran larvae and small Araneae. Arthropods were found to be extremely scarce in birch during the period when birds were seen to forage there.

Amino acid analyses were performed on samples of blue tit eggs, Araneae and Lepidopteran larvae collected at three times through the laying season. These analyses indicated that the sulphur-containing amino acids, methionine and cysteine, would most likely be the most limiting essential amino acid for a laying blue tit consuming an insectivorous diet.

In the first supplementary feeding experiment, one group received a pure energy food (animal fat) whilst the other received cooked eggs (on the assumption that this would contain all the necessary nutrients for egg formation). Supplementary lipid and supplementary egg resulted in the same degree of laying enhancement compared with control birds. However, the provision of supplementary egg resulted in a significant increase in egg volume (approx. 7%) whilst supplementary lipid had no significant effect on egg size. The eggs laid early in the laying sequence showed the greatest size increase, with eggs from the egg-fed treatment group being significantly larger than those in both the fat-fed and control groups. I discuss the

hypothesis that energy supply may influence the initiation of laying, whilst specific nutrients may proximately constrain egg production.

The hypothesis that egg production may be proximately constrained by the availability of high quality protein was examined in a second supplementary feeding experiment. This involved the provision of supplementary foods of identical calorific, 'crude' protein and non-protein content, but differing amino acid composition. Both supplementary diets resulted in a similar degree of laying advancement compared with control birds; supporting the hypothesis that initiation of laying is influenced by energy supply. Clutch size declined significantly through the season in all three experimental groups and, after controlling for laying date, mean clutch size was found to be significantly larger in the treatment group receiving 'high-quality' protein compared with those receiving 'low-quality' protein or controls; whilst in the treatment group receiving 'low-quality' protein, mean clutch size did not differ significantly from control. The increased clutch size in the 'high-quality' protein treatment group was reflected in a higher mean number of fledglings compared with control, although this difference was not statistically significant. The hypothesis that egg production may be proximately constrained by the availability of specific essential amino acids is discussed and considered in relation to our existing knowledge regarding the role of food supply in the determination of clutch size in the blue tit.

Simple balance calculations of essential amino acid and calorific content in arthropods compared to estimated requirements for laying, suggested that the intake level needed to satisfy energy requirements would be about four times greater than that which would lead to amino acid limitation. However, such calculations may be a significant misrepresentation of the nutritional scenario experienced by laying blue tits, particularly those breeding early in oak-dominated woodland. Arthropods may be extremely scarce and it is hypothesised that the diet may contain a high proportion of low protein quality plant tissue and high calorific content plant secretions such as sap and nectar; leading to the availability of specific amino acids forming a proximate constraint on egg formation.

Finally, calcium for eggshell formation has been proposed as a potentially important nutritional constraint on egg production in locations where acid precipitation and underlying geology combine to make natural calcium sources scarce. Study areas were chosen which receive amongst the highest levels of acid precipitation in the UK and have particularly poorly buffered soils with amongst the lowest calcium levels in the UK. A survey of snail abundance (the most important natural calcium source for tits) revealed that they were extremely scarce in this location. However, the experimental provisioning of breeding blue tits with supplementary calcium sources provided no evidence that egg production was in any way constrained by calcium availability.

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# CHAPTER 1

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*A bird a'flight*
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Spike Milligan

# General Introduction

## 1.1 Nutritional requirements for egg production

Egg production is a nutritionally demanding process for many birds. In some species, a clutch of eggs representing more than the female's own body weight may be produced over the course of only a few days. The estimated peak daily energy requirement for egg production ranges from 29% of basal metabolic rate (BMR) for raptors and 13 - 41% in some passeriformes, up to 160 - 216% in some waterfowl and the kiwi, *Apteryx australis* (Walsberg 1983; Ricklefs 1974; Meijer *et al* 1989). Whilst the energetics of egg production have been comparatively well studied, the coincident requirement for protein has received considerably less attention. Robbins (1981, 1993), noted that the estimated increase in daily protein requirement for egg production varied from 72% to 237% of maintenance requirements for different avian groups. The long-standing emphasis on the energetics of egg production may, therefore, have neglected an equally important problem; that of obtaining sufficient protein.

These demands, above the normal requirements, vary from species to species depending on the clutch size and relative size of the egg and the rate at which they are laid. In interspecific comparisons, average egg size is inversely proportional to body weight - that is, smaller birds generally lay larger eggs relative to body weight than do larger birds (Lack 1968; Rahn *et al* 1975). Variation in egg composition between species largely reflects the differing stage of embryonic development at hatching (Sotherland & Rahn 1987); the eggs of precocial species having larger yolks than those of altricial birds. As the yolk contains virtually all of the egg's lipid, increasing the relative size of the yolk increases the amount of energy per unit of egg. Differences in energy density are, in fact, even larger than suggested by relative yolk size because the yolk lipid concentration is, on average, 41% higher in the eggs of precocial species (~37% of fresh yolk) than in altricial species (~26% of fresh yolk) (Ricklefs 1974; Roca *et al* 1982). Consequently, in relation to body size and energy requirements for egg production, relative protein requirements would be expected to be greatest in small, altricial species producing large clutches.

Aside from yolk and albumen, the other primary component of eggs is the shell. Avian eggshell is predominantly composed of calcium carbonate (~90%) of which calcium forms 40%. Consequently, the calcium requirements of laying birds are many times higher than those for normal body maintenance (Robbins 1993; Scott, Nesheim & Young 1982; Taylor & Moore 1954; Simkiss 1975).

## 1.2 Source of the nutrients for egg production

The nutrients required for egg synthesis may either be derived exogenously, by increasing dietary intake, or endogenously, by drawing upon body reserves. In addition, a reduction in the daily energy or protein metabolism of the female can allow her to divert a greater proportion of resources into egg formation (Walsberg 1983; Houston, Dounan & Jones 1995a; Drent & Daan 1980).

Within the species that have been investigated, an array of strategies have been found, ranging from total reliance on body reserves to complete dependence on dietary intake, with no detectable use of reserves (Houston *et al* 1995c). For example, species which do not feed at all during laying, like the Adélie penguin (*Pygoscelis adeliae*, Astheimer & Grau 1985), or have very limited access to food during laying, such as some Arctic-nesting geese (Ankney & MacInnes 1978; Raveling 1979; but see Choiniere & Gauthier 1995), may rely entirely on endogenous sources for all the eggs' nutrients. In contrast, species such as the brown-headed cowbird (*Molothrus ater*, Ankney & Scott 1980) and white-bellied swiftlet (*Collocalia esculenta*, Hails & Turner 1985) appear to utilise virtually no endogenous reserves for egg production. For many species the situation is somewhat intermediate, with reserves being used to augment the dietary intake (e.g. Ojanen 1983; Jones 1987a; Krementz & Ankney 1986).

Although lipid is perhaps the most ubiquitous form of reserve, several species studied have been found to utilise endogenous protein reserves to compensate for inadequate protein in the diet (e.g. Jones & Ward 1976; Houston *et al* 1995a,b,c; Ankney 1984; Raveling 1979a,b). Further, it has been suggested that some other species, apparently using only lipid reserves, do so in order to allow selective foraging for protein rich food items (e.g. Drobney 1980; Krapu 1981). Studies on a number of species have concluded that protein, rather than energy, might be the limiting factor for egg production (Jones & Ward 1976; Raveling 1979a; Fogden & Fogden 1979; Houston, Jones & Sibly 1983; Drobney & Fredrickson 1985; Bolton, Houston & Monaghan 1992).

The extent to which birds depend on endogenous reserves for meeting the energy and nutrient demands of egg production obviously varies among species, and body size may be one of the main determinants. The metabolic demands of larger birds are relatively lower than those of smaller birds (Walsberg 1983), so that, in comparison to small birds, they can store relatively more energy for the same proportion of body weight. Moreover, an upper limit on the level of endogenous reserves may exist due to the severe costs of extra weight on flight efficiency (e.g. Freed 1981; Norberg 1981). Consequently, small species may have to depend on exogenous resources much more than do larger species (Ankney & Scott 1980; Jones & Ward 1979). Further, it has been proposed by Murphy (1986) that it may be additionally

disadvantageous for some smaller birds to have large amounts of muscle. Muscle is a very metabolically active tissue and, consequently, birds with less muscle may be able to divert a greater portion of daily energy intake to reproduction (Wiley 1974; Downhower 1976).

It has been suggested, however, that in some species muscle may make selective contributions, providing essential amino acids that are limiting in the diet at the time of egg production (Houston *et al* 1995a,b,c; Fogden & Fogden 1979; Kendall *et al* 1973; Krementz 1984). Whilst not accounting for a substantial quantitative amount of total protein, it may still make an important qualitative contribution. The amino acid composition of egg proteins is somewhat unusual. In particular, egg proteins have levels of the sulphur amino acids cysteine and methionine which are often greater than those found in plant proteins and some animal proteins (Harvey 1970).

As stated earlier, birds require large amounts of calcium for effective eggshell formation. Some large species are able to mobilise skeletal calcium for this purpose, but in small passerines, this can provide only a small fraction of that required for the clutch (Ankney & Scott 1980; Graveland & Van Gijzen 1994; Schifferli 1979; Houston *et al* 1995a; Graveland & Berends 1997). In species laying large clutches, the calcium content of the eggs can approach, or exceed, that of the female's entire skeleton (Maclean 1974; Perrins 1979; Jones 1976; Ormerod *et al* 1988). Most small birds, therefore, must depend upon obtaining sufficient calcium from their diet.

### 1.3 Nutritional constraints on egg production

In order to raise young successfully, birds are generally believed to adjust their breeding decisions ultimately to food availability (Lack 1968). In many temperate species, breeding occurs during a relatively short period of food abundance in spring (Murton & Westwood 1974). Observational correlative studies have often demonstrated an association between the natural food abundance and reproductive parameters such as onset of laying date (Perrins 1965; Newton 1976; Lundberg *et al* 1981; Dijkstra *et al* 1982; Murphy 1986; Blancher & Robertson 1987; Nager & van Noordwijk 1995), egg size (Nisbet 1973; Murton & Westwood 1974; Otto 1979; Murphy 1986; Nager & Zandt 1994) and egg number (Perrins 1965; Bryant 1975; Newton 1976; Lundberg *et al* 1981; Dijkstra *et al* 1982; Hussell & Quinney 1986; Murphy 1986; Blancher & Robertson 1987).

However, food availability and reproductive parameters may be varying independently in response to other environmental factors. Better evidence for a causative relationship between food supply and reproductive performance comes from supplementary feeding studies. These

have produced mixed results, however (reviews in Martin 1987; Arcese & Smith 1988; Boutin 1990; Aparicio 1994); many succeeded in advancing laying date, but far fewer resulted in any increase in egg size or number. Several hypotheses have been proposed to account for these variations between studies.

Firstly, some of the variation in results can possibly be ascribed simply to differences in the life history strategies of the various species examined. For example, in some colonial breeding species any advantage of advancing breeding in response to food provisioning may be outweighed by the disadvantages of becoming asynchronous with other individuals in the colony (Robertson 1973; Hoogland & Sherman 1976). Similarly, Svensson (1995) provided evidence that multi-brooded birds tend to advance their laying dates to a greater extent following food provisioning than single-brooded birds. However, this does not adequately explain much of the variation between different studies of the same or similar species.

Secondly, the relationship between food availability and reproductive traits may be moderated by other factors, which become limiting at high food availability (Martin 1987; Arcese & Smith 1988; Boutin 1990; Schultz 1991; Källander & Karlsson 1993; Wiebe & Bortolotti 1994; Svensson & Nilsson 1995; Nager, Rüegger & van Noordwijk 1997). Supplementary food would then affect only populations, or individuals within a population, when natural food availability fell below a critical threshold level.

Finally, most experimental studies have focused on the energy content of the diet, and few studies have considered the nutritional quality of the supplementary food. However, as we have already established, egg formation is demanding, not only in terms of energy, but also in terms of nutrients such as protein. Differences in the nutritional quality of the supplementary food used could result in different responses by the experimental subjects. For example, Smith *et al* (1980) found only an advancement of laying date in song sparrows (*Melospiza melodia*) supplemented with millet seed. However, in a later experiment on the same population, using a higher quality supplementary food, all the breeding parameters examined were enhanced with the result that four times more young were produced in experimental territories than controls (Arcese & Smith 1988). In both years, environmental conditions and breeding densities were reported to be similar.

It has further been suggested that, as a result of the somewhat unusual protein composition of avian eggs, egg production may be limited by the availability of specific essential amino acids in the diet (Magrath 1992; Bolton, Houston & Monaghan 1992). A field study by Bolton *et al.* (1992) on the lesser black-backed gull (*Larus fuscus*), involving the provision of supplementary foods of differing nutritional composition, but equivalent calorific value, during the pre-laying period, demonstrated that egg production could be differentially affected by the protein quality of the supplementary food provided. Experimental work with

captive zebra finches (*Taeniopygia guttata*) has further demonstrated the importance of diet quality on egg production and suggests that an imbalance in the amino acid composition of egg proteins compared to those present in the diet may result in access to quality protein having an important influence on egg production (Houston *et al* 1995a).

## 1.4 The study species

The Paridae are a family of small passerine birds, belonging to the sub-order Oscines. Two members of this family, the blue tit, *Parus caeruleus*, and its relative the great tit, *Parus major*, have, perhaps, been more intensively studied than any other species of wild bird. In Scotland, the blue tit is the more common of the two, and was chosen as the study species for this investigation.

The blue tit is distributed over much of Europe and western Russia (Perrins 1979). In Britain, the indigenous population has been recognised as a distinct subspecies, *Parus caeruleus obscurus*, in contrast to *P. caeruleus caeruleus*, on the continental mainland.

Although found in a variety of habitats, such as parks, gardens and hedgerows, the blue tit is essentially a bird of deciduous woodland, especially oakwoods. It will readily nest in nestboxes, if provided, although Gibb (1950) estimated that only about 70% of the breeding blue tit population in Marley wood, near Oxford, utilised them, despite abundant availability.

Blue tits generally lay one egg per day, with an average clutch size, in Britain, of around 10 - 12 eggs (Perrins 1979; Cramp & Perrins 1993); amongst the largest average clutch size of any altricial bird. Genuine second broods are extremely rare in Britain (Gibb 1950; Perrins 1979).

The blue tit will feed on a wide variety of food types, including seeds, nuts and fruit, but primarily takes insects and spiders when available (Cramp & Perrins 1993). The diet often reflects local and seasonal changes in food abundance. Throughout the majority of the breeding season, the diet consists almost entirely of invertebrates. Lepidopteran larvae can form over 91% of the diet of a brood in oak woodland (Betts 1955), and the pair can make up to 1000 visits per day, depending on brood size (Nur 1984c).

The breeding success of tits is heavily dependent upon them being able to raise their young within the narrow time window of peak caterpillar availability (Perrins 1965; Perrins & McCleery 1989; Perrins 1991; van Noordwijk, McCleery & Perrins 1995). Pre- and post-fledging survival and establishment success of juveniles is often negatively related to hatching date (Norris 1993; Perrins 1979; Nilsson & Smith 1988; Nilsson 1989, 1990; Perrins 1970).

Consequently, females are often under strong pressure to lay early (van Noordwijk *et al* 1995). Early in the season, however, food availability may be relatively low and females may potentially, therefore, face a nutritional constraint regarding how early they are able to lay and/or the level of resources they are able to invest in a clutch.

## 1.5 Study sites

The majority of the field work for this study was carried out between 1994 and 1996 in an area of woodland surrounding the University Field Station at Rowardennan; located on the eastern shores of Loch Lomond, in west-central Scotland (see figure 1). In 1994, additional fieldwork was carried out at Tarbet and Inversnaid, which are also located on the perimeter of Loch Lomond. The predominant habitat at all sites is oak dominated deciduous woodland. 197 nestboxes were deployed in the woodland at Rowardennan in 1991/92, and a further 76 were deployed in the winter of 1993. 66 nestboxes were available at the site in Tarbet and 53 at Inversnaid.

## 1.6 Aims and layout

The focus of this study was to investigate potential nutritional constraints on egg production in the blue tit. Particular investigative emphasis was placed on the role of protein nutrition, a previously somewhat neglected avenue of research.

*Chapter 2* examines the foraging locations and potential prey available to blue tits at the study site during the egg formation period. Amino acid analyses of blue tit eggs and potential prey were used to indicate which essential amino acids one would expect first to become limiting.

The influences of food availability and quality in determining both the timing and level of investment in egg production were investigated through supplementary feeding experiments, described in *Chapters 3 and 4*. By providing food supplements which differed in nutritional composition, but were of consistently high calorific content, the hypothesis that egg production may be constrained by protein supply was tested in 1994. The importance of protein quality was further examined, in 1995, by providing supplements of equivalent calorific and crude

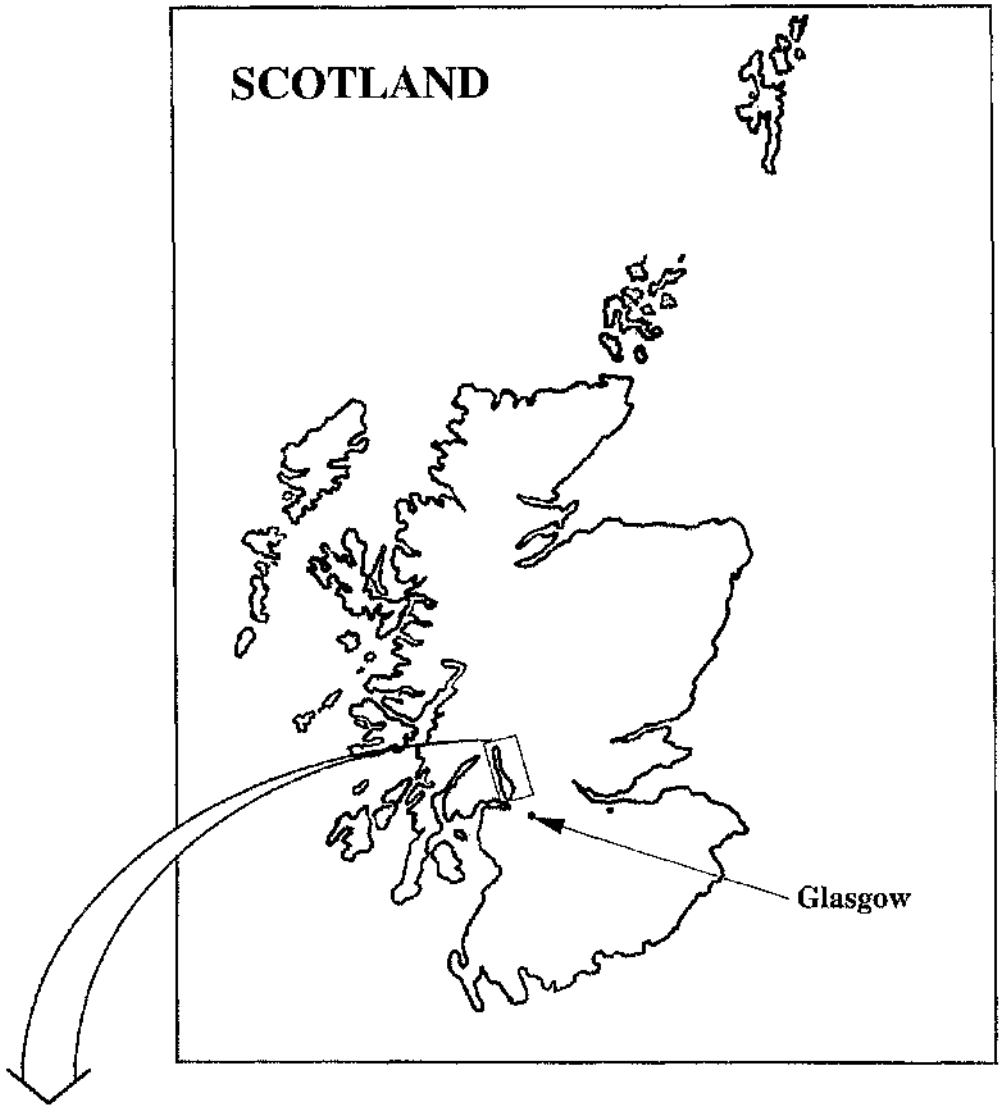
protein content, but differing amino acid composition, in order to investigate the hypothesis that specific amino acids may be scarce in the diet at the time of egg formation.

In *Chapter 5*, the availability of calcium for eggshell formation as a potential constraint on reproduction is examined in a calcium supplementation experiment. Potential declines in environmental calcium availability as a result of anthropogenic acid deposition are considered.

*Chapter 6* brings together several years of laying data for the study population. This allows the previous experimental results to be placed within the context of longer term natural variation in the study population. In addition, variation in nestbox site quality is investigated and the possibility of using this information to enhance the analysis of experimental data considered.

In the 'General discussion' (*Chapter 7*), the findings of previous chapters are integrated and an evaluation made regarding the nature and identity of nutritional constraints on egg production in the blue tit.

N.B. The amino acid analyses detailed in *Chapter 2*, were performed relatively late in the course of this research. The findings, therefore, were not available when designing the experiments detailed in *Chapters 3 & 4*.



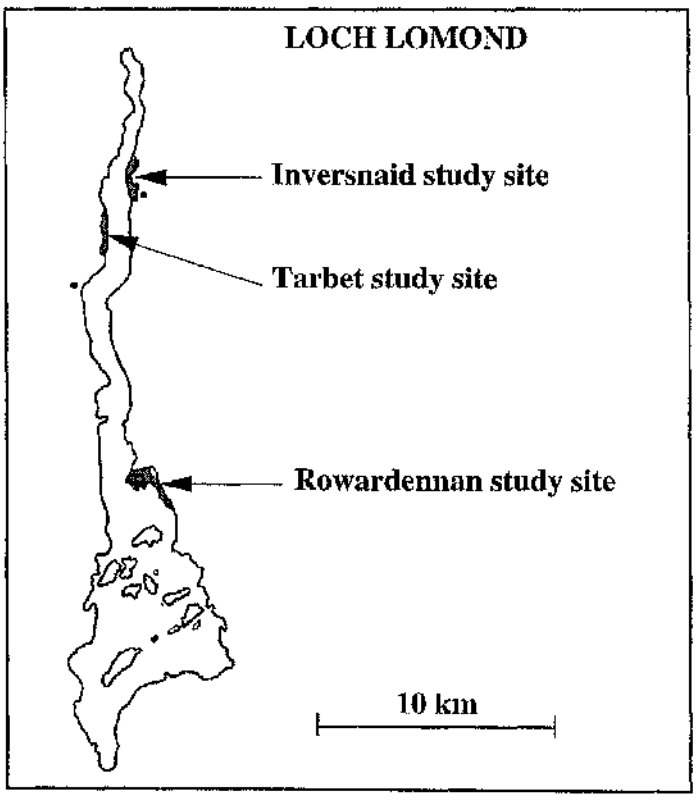




**Figure 1**: Map of study sites

# CHAPTER 2

## Foraging locations, food availability and amino acid composition of diet prior to and during the egg laying period.

*"Bees make honey and we just accept it.....but do spiders make gravy; do earwigs make chutney?"*

Eddie Izzard

## 2.1 Introduction

Food abundance and quality are major factors influencing the timing of breeding and the level of investment in a breeding attempt (Martin 1987; Arcese & Smith 1988; Ankney & Alisauskas 1991; Bolton, Houston & Monaghan 1992) and, consequently, can determine the nature of food items taken by breeding birds (Krapu & Swanson 1975; Jones & Ward 1976; Ankney & Scott 1980; Krementz & Ankney 1988). For many species, the most successful breeders are those that raise their young when food is most plentiful (Perrins 1970; Arcese & Smith 1988; Simons & Martin 1990; Richner 1992; Verhulst 1994; Nager & van Noordwijk 1995; van Noordwijk, McCleery & Perrins 1995) and, especially amongst Parids, post-fledging survival and establishment success of juveniles is often negatively related to hatching date (Perrins 1979; Nilsson & Smith 1988b; Nilsson 1989, 1990). Thus, in many years, there is strong selection pressure for individuals to lay early, when food is still relatively scarce (van Noordwijk *et al* 1995).

The nutrient requirements of laying birds comprise those necessary for routine body maintenance, plus those required for egg synthesis. The principal constituents of avian eggs, in addition to water, are protein, lipid and calcium and limited availability of any of these may constrain egg production (Houston 1978; Drobney & Frederickson 1985; MacLean 1974; Bolton, Houston & Monaghan 1992; Houston, Donnan & Jones 1995a).

A number of avian species have been shown to selectively forage for protein-rich food items around the time of laying (Ankney & Scott 1980; Krapu & Swanson 1975; Krementz & Ankney 1988; Jones & Ward 1976; Krapu 1981; Drobney & Frederickson 1979). Protein, however, is a complex macronutrient composed of around twenty common amino acids, ten of which cannot be synthesised in the body and are, therefore, essential components of the diet. The nutritional value of a food item thus depends on both the quantity and quality of protein contained therein. Protein quality is defined in terms of the available essential amino acids in the protein relative to the essential amino acid requirements of the animal; proteins supplying an array of amino acids that closely parallel the animal's needs are considered high quality (Murphy 1994). Research on zebra finches (Selman & Houston 1996) and lesser-black-backed gulls (Bolton, Houston & Monaghan 1992) suggests that avian egg production may be constrained by the availability of high quality protein. Further, there is some evidence that birds can discriminate between foods on the basis of their protein quality (Murphy & King 1987).

This chapter focuses, firstly, on the identity and nutritional composition of the diet of blue tits around the time of laying and, secondly, on their nutritional requirements for maintenance and egg production

Extensive research was carried out in southern England in the 1950's, to investigate foraging niche separation in Paridae through the year. Betts (1955) examined the gizzard contents of four species (blue tits, great tits, coal tits and marsh tits), whilst Hartley (1953) and Gibb(1954) studied their feeding biology by direct observation. These studies have provided a great deal of valuable information on foraging locations and diet composition. They did not, however, fully elucidate the effects of relative tree species abundance on tree choice, making it difficult to extrapolate their results, with confidence, to a woodland, such as ours, of differing tree species composition. Also, the difference in climate between southern England and central Scotland, although not extreme, is sufficient to cause plant development, bud burst and average laying date of blue tits, to occur some time later in Scotland. Further, such differences in climate can influence the species composition of invertebrates occurring in any particular tree species. Thus, an observational survey of blue tit foraging locations was carried out and systematic sampling performed concurrently, at identified foraging niches, in order to provide additional information regarding the identity and availability of potential food items.

I report here:

1) The foraging locations of blue tits prior to and during the egg laying period.

2) The identity and availability of potential invertebrate prey at identified foraging locations.

3) The amino acid composition of blue tit eggs in relation to those for other avian species.

4) The amino acid composition of prey items that may be taken by tits.

5) Which amino acid(s) might first become limiting during egg production

## 2.2 Observations of foraging blue tits

### 2.2.1 Methods

Observations of foraging blue tits, prior to and during the laying period, were made between 15th April 1996 and 28th May 1996 (Mean date of laying for birds in this year was 9th May). I walked through the study area constantly scanning for blue tits. When a blue tit was spotted, I stopped and recorded information on: i. whether the bird was situated on the ground or in a tree, ii. species of tree, iii. site within tree (trunk, branch, twig, leaf or bud), iv. activity. Once this information was recorded, I continued walking. I followed a fairly linear route and this, in conjunction with a fast walking speed, made multiple observations of the same individual within one observation session unlikely.

### 2.2.2 Results

No systematic survey was made of the distribution and relative abundance of different tree species within the whole study site. However, a brief census of all trees in a representative area of woodland contained about 85% oak, 10% birch and 5% other species including alder, holly and ash.

About 30 hours of observations of foraging tits were made and 302 records were obtained. Only 2 records of blue tits foraging on the ground were obtained, in the period 15 - 29th April. The distribution of records between different tree species from mid April to late May, is shown in Figure 2.1. Chi-square analysis (after grouping together 'birch', 'other trees' and 'ground' categories) showed a significant difference in foraging site use between early, mid and late date periods ($\chi_2^2 = 24.47$, $P < 0.01$); primarily due to an increasing dependence on oak trees as the season progressed. Information on the position of blue tits within trees is presented in Figure 2.2. In this figure, records of birds at buds, leaves, catkins and twigs have been grouped together. Although the identity of these various sites obviously differs, they are all essentially the same spatial zone of the tree, and their identity changes only with the progress of the season and the concurrent development of the tree. Since the primary reason for this observational survey was to identify the appropriate zones for invertebrate sampling, the information presented in Figure 2.2 is sufficient, and clearly shows the preference shown by tits for the outer twigs on the tree.

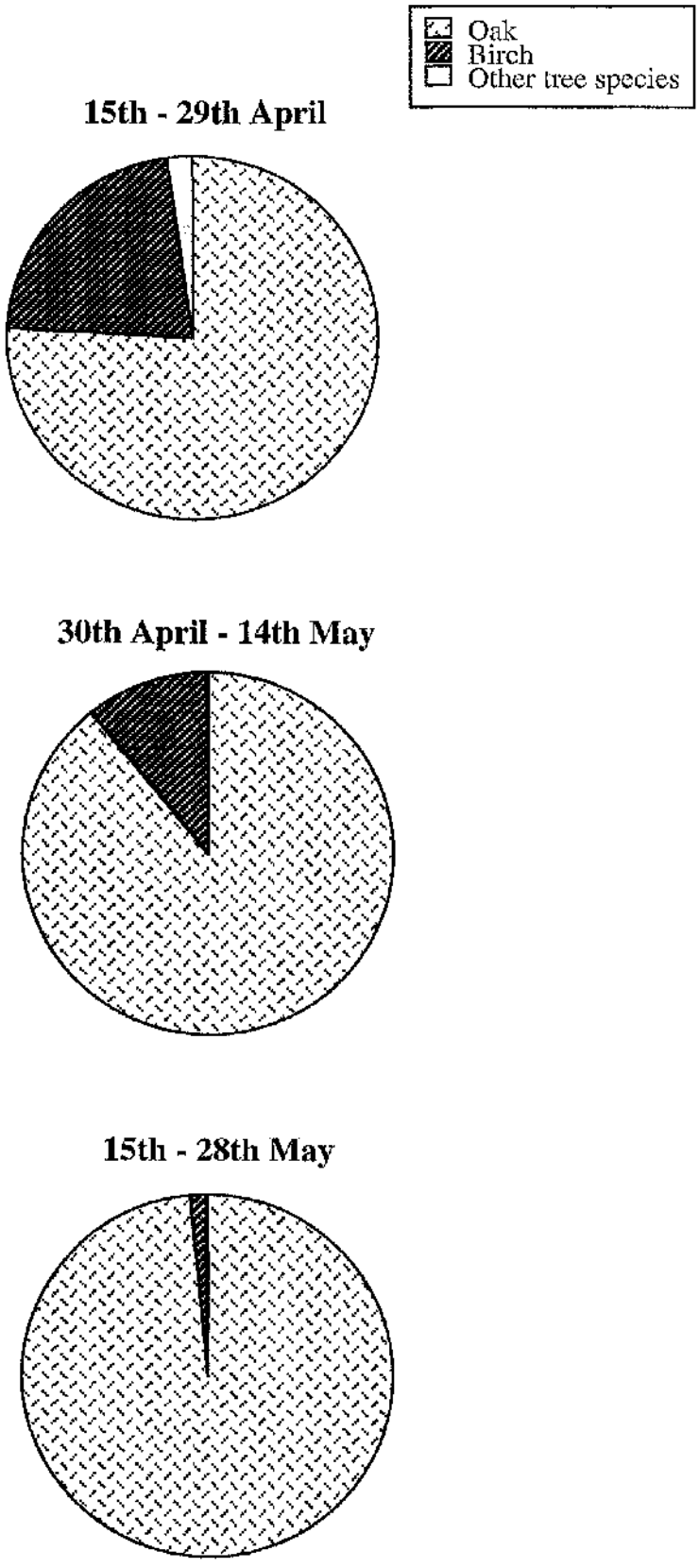



**Figure 2.1**: Proportion of foraging by Blue tits observed in different tree species (from 300 observations).

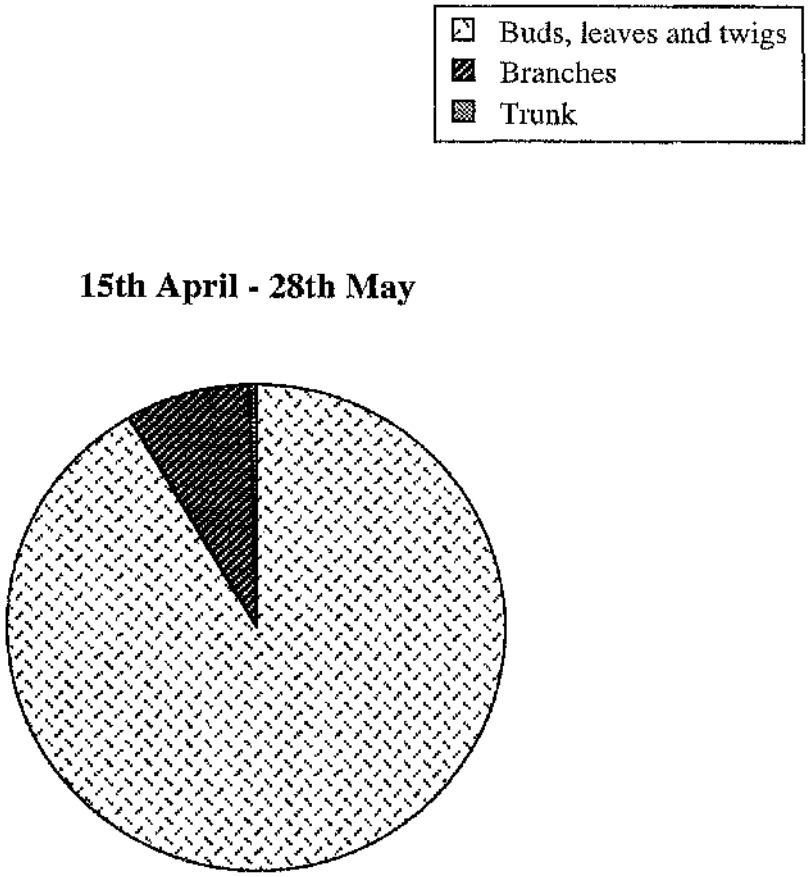



**Figure 2.2**: Proportion of foraging by blue tits observed in different sections of trees (from 300 observations)

## 2.3 Arthropod abundance at blue tit foraging sites prior to and during the egg laying period

### 2.3.1 Methods

Oak trees were sampled systematically at weekly intervals throughout the period 29th April to 20th May 1995, and 25th April to 24th May 1996. Birch trees were only sampled in 1996 and only during the weeks when blue tits were seen to forage on them.

OAK: In order to ensure that trees were sampled in an unbiased fashion, random number tables were used to select the identity numbers of six nestboxes. The closest oak tree to each of these boxes was then sampled. Sampling involved snipping off the terminal 30 cm (approx.) from a branch, using a 3m long tree pruner, which was then caught on a beating tray held below the blades of the pruner. The severed twig was placed in a labelled polythene bag and any invertebrates which had been dislodged from the twig and were on the beating tray were collected and placed in a labelled container. Four branch ends, from different aspects, were collected per tree. All samples were taken back to the laboratory and stored in a refrigerator until they were examined. Each twig was placed individually in a white plastic tray and any invertebrates found on it were collected, identified and recorded. Any buds or partially unopened leaves were dissected to check for invertebrates within, since it is known that blue tits will tear open buds to get at such prey. All samples were processed within one day of collection.

BIRCH: Birch trees were sampled in each of the weeks during which blue tits were observed foraging at birches. Sampling involved snipping off the terminal 30 - 50 cm of branches, using a 3m long tree pruner, then catching them on a beating tray held below the blades of the pruner - as with the oak trees. Branch ends were taken from different aspects of six randomly chosen individual trees. Sampling was continued until a plastic sack (approx. volume, 60 l) had been filled with birch twigs. These were then taken back to the laboratory for examination. In the laboratory, twigs were placed in a white plastic tray and examined systematically. Any invertebrates found were collected, identified and recorded. Any buds, partially unopened leaves, or catkins were dissected to check for invertebrates within.

### 2.3.2 Results

OAK: The abundances of potential prey items found on the oak samples, at weekly intervals through the sampling period, are presented in Figures 2.3a and 2.3b and Tables 2.1a and 2.1b, for 1995 and 1996, respectively. The data presented in these figures are the mean number of items per tree for the sum of the four branch samples.

**Table 2.1a:** Mean (± S.E.) prey abundance per tree, on four terminal oak branches, at weekly intervals through the laying period (1995).

| | 29TH April | | 7TH May | | 13TH May | | 20TH May | |
|---|---|---|---|---|---|---|---|---|
| Lepidoptera | 10.33 | ±4.21 | 21.75 | ±5.70 | 27 | ±9.58 | 42.38 | +16.96 |
| Araneae | 0.33 | ±0.21 | 1.25 | ±0.41 | 0.88 | ±0.23 | 0.5 | ±0.27 |
| Coleoptera | 0 | ±0 | 0.25 | ±0.16 | 0.38 | ±0.26 | 0.62 | ±0.26 |
| Hemiptera | 0.50 | ±0.50 | 0.5 | ±0.19 | 1.12 | ±0.35 | 0.25 | ±0.16 |
| Acari | 0 | ±0 | 0 | ±0 | 0.88 | ±0.74 | 0 | ±0 |
| Psocoptera | 0 | ±0 | 0 | ±0 | 0 | ±0 | 0 | ±0 |
| Other larvae | 1.00 | ±0.52 | 0 | ±0 | 0.12 | ±0.12 | 0 | ±0 |
| Leaf galls | 2.5 | +2.5 | 9.25 | ±2.23 | 12 | ±3.88 | 12.75 | ±2.17 |

**Table 2.1b:** Mean (± S.E.) prey abundance per tree, on four terminal oak branches, at weekly intervals through the laying period (1996).

| | 25TH April | | 2ND May | | 9TH May | | 16TH May | | 24TH May | |
|---|---|---|---|---|---|---|---|---|---|---|
| Lepidoptera | 0 | ±0 | 1.50 | ±0.62 | 10.00 | ±2.82 | 47.00 | ±13.41 | 84.00 | ±19.36 |
| Araneae | 0 | ±0 | 0.17 | ±0.17 | 0.83 | ±0.31 | 1.50 | ±0.43 | 8.00 | ±1.55 |
| Coleoptera | 0 | ±0 | 0.50 | ±0.23 | 0.17 | ±0.17 | 0 | ±0 | 3.00 | ±0.89 |
| Hemiptera | 0 | ±0 | 0 | ±0 | 0 | ±0 | 0.50 | ±0.50 | 0.500 | ±0.30 |
| Acari | 0 | ±0 | 0 | ±0 | 0 | ±0 | 0.17 | ±0.17 | 0 | ±0 |
| Psocoptera | 0.50 | ±0.22 | 0.50 | ±0.34 | 0 | ±0 | 0 | ±0 | 0 | ±0 |
| Other larvae | 0 | ±0 | 0 | ±0 | 0 | ±0 | 0 | ±0 | 0 | ±0 |
| Leaf galls | 0 | ±0 | 0 | ±0 | 0 | ±0 | 7.50 | ±1.57 | 6.00 | ±1.22 |

The mean masses of individual caterpillars, on the sampled branches, at approximately weekly intervals in 1996 are presented in Table 2.1. For the sake of accuracy and convenience, mean mass was obtained by weighing a sample of caterpillars and then dividing by the total number of individuals in the sample, rather than weighing individual caterpillars and then taking an arithmetic mean. A reliable mean mass for caterpillars earlier in the season was not obtained due to their scarcity and very small size. Therefore, the values obtained were plotted on a graph and a second order polynomial fitted, Figure 2.4. Using the equation given, an estimated mean mass for caterpillars on 2nd May was calculated as 7.55mg. Using the data for caterpillar abundance and caterpillar mass, we can calculate an index of relative biomass (abundance × mean individual mass) for each week during the egg laying period. This is presented in Figure 2.5 for 1996.

**Table 2.2:** Mean mass of caterpillars at approximately weekly intervals.

| Date | | Mean fresh caterpillar mass (mg) | (n) |
|---|---|---|---|
| 9th | May 1996 | 9.49 | (33) |
| 16th | May 1996 | 13.67 | (49) |
| 24th | May 1996 | 22.38 | (61) |
| 7th | June 1996 | 41.89 | (72) |
| 16th | June 1996 | 61.35 | (50) |

BIRCH: In the first week of sampling (26th April), no invertebrates were found on any of the birch twigs examined. In the second week (3rd May), sampled twigs contained 2 Coleoptera larvae and 1 sawfly larva found inside catkins, and 3 very small Lepidopteran larvae, 4 adult Coleoptera and 1 unidentified nymph were found on the surface of the catkins and buds. Invertebrate numbers on birch at the time of egg laying were clearly extremely low, and I have not considered the invertebrates on birch any further.

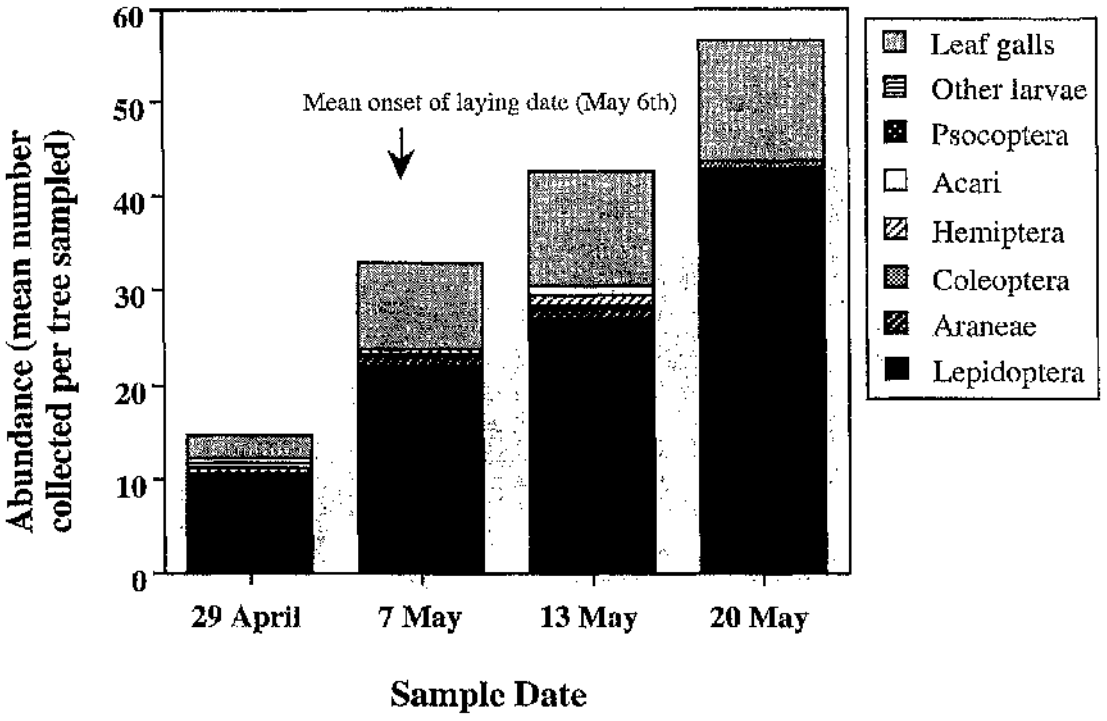



**Figure 2.3a**: Mean prey abundance per tree ($n$=6), on four terminal oak branches, at weekly intervals through the laying period (1995).

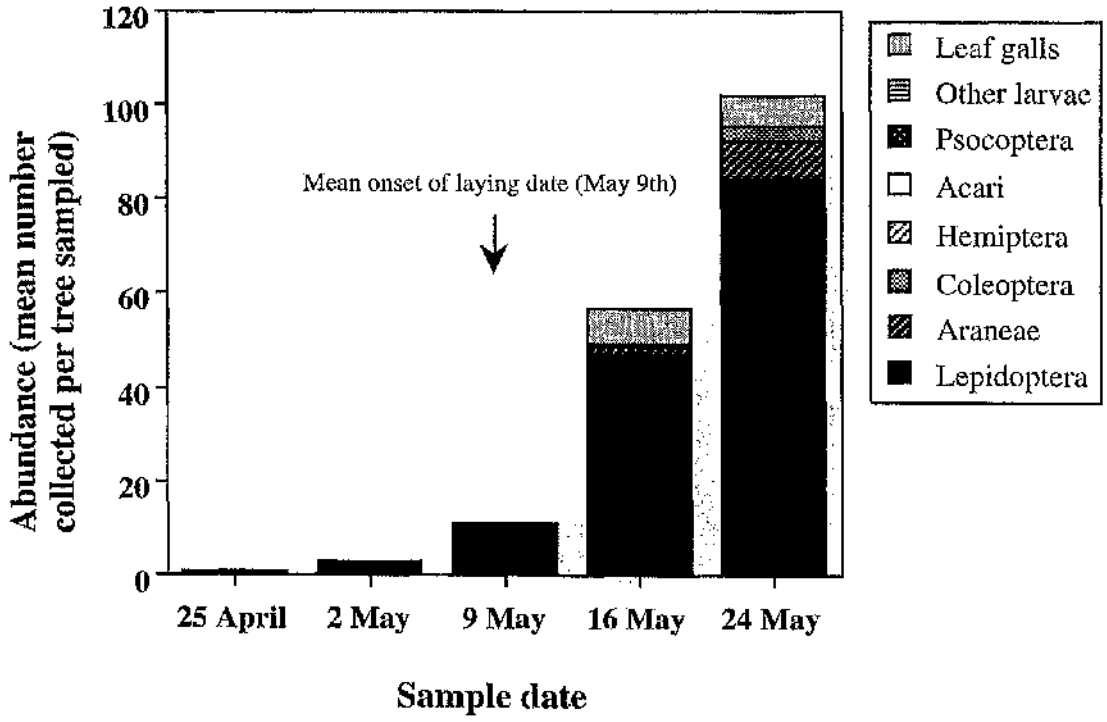



**Figure 2.3b**: Mean prey abundance per tree ($n$=6), on four terminal oak branches, at weekly intervals through the laying period (1996).

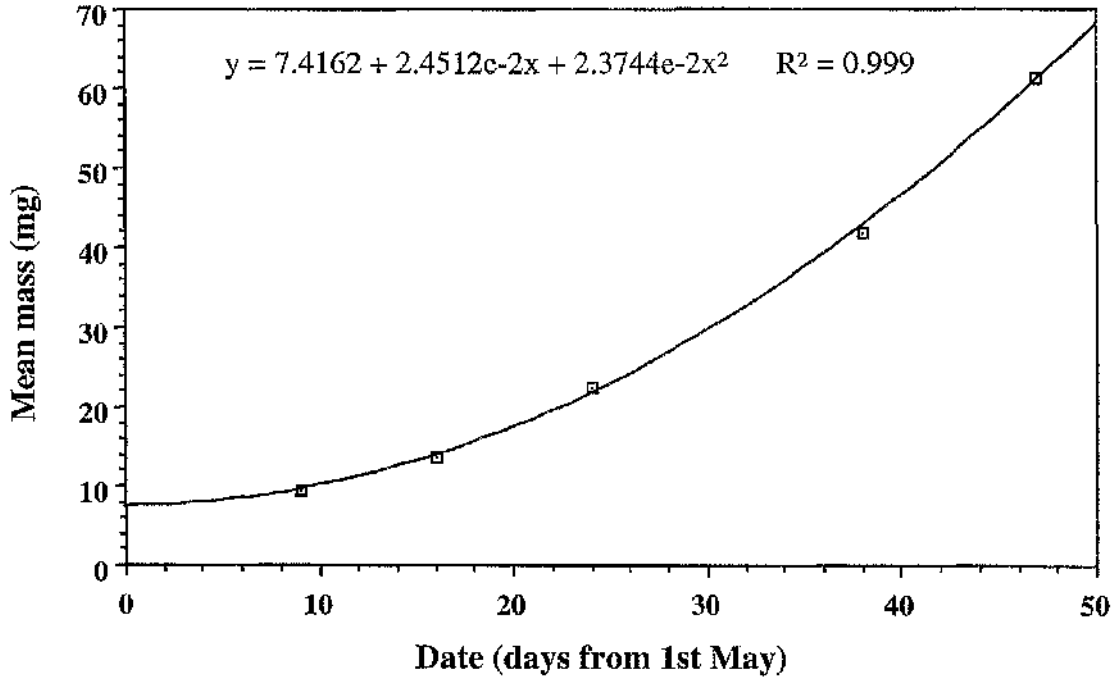



**Figure 2.4**: Mean mass of individual caterpillars at weekly intervals (1996).

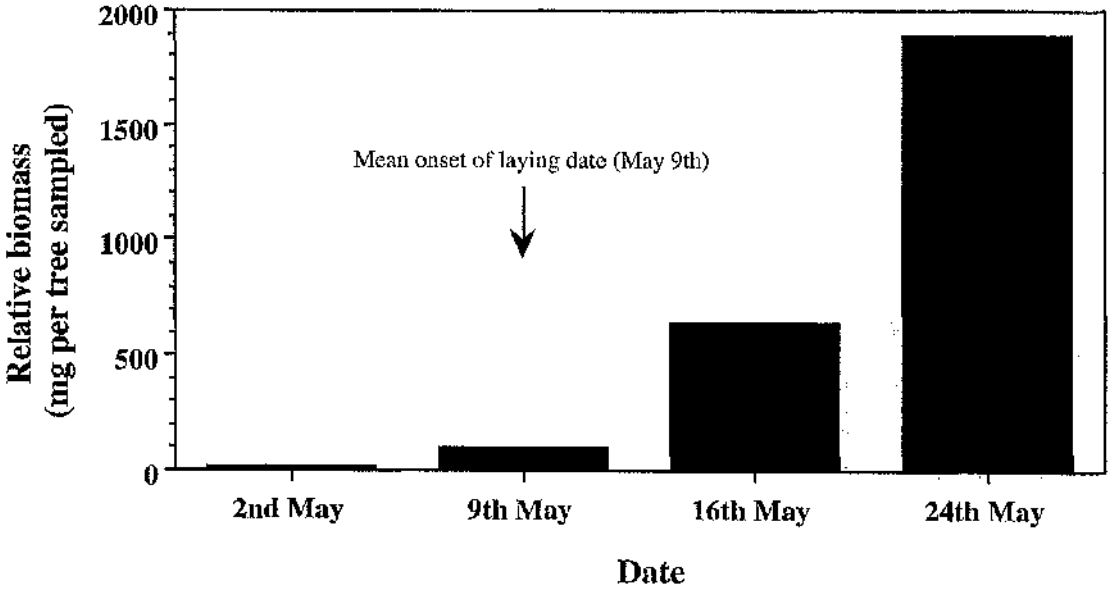



**Figure 2.5**: Relative biomass of caterpillars, on four sampled twigs per tree, through the laying period (1996).

## 2.4 Discussion I

Blue tits were observed foraging predominantly at oak trees early in the season and as the season progressed they foraged almost exclusively on oak. A preference for oak has also been found in other studies of blue tit foraging, although it did not account for such a large proportion of foraging sites as was found here (Gibb 1954; Hartley 1953). This can be explained, however, by the fact that these previous studies were conducted in considerably more mixed woods, with a lower overall proportion of oak trees.

Within trees, virtually all foraging occurred at the outer extremities of branches - twigs, buds, leaves or catkins, depending on tree species and exact time of season. Again, this largely confirms the findings of other studies (Gibb 1954; Hartley 1953; Betts 1955). Blue tits are considerably lighter than great tits (the other common Parid of deciduous woodland) and generally considered to be more agile. They are thus able to exploit the outer reaches of trees; often hanging upside down from leaves or twigs in order to forage; whilst great tits concentrate more on the stouter branches, trunk and ground.

Once confirmed by observation, the foraging locations of blue tits were sampled and analysed periodically in order to determine the potential prey available there. Perhaps the most striking finding was the apparent scarcity of any invertebrates, immediately prior to and during the early stages of the egg laying period. Whether egg production and onset of laying are actually directly constrained by food availability or not, it would appear that invertebrate prey in our study area was certainly in relatively short supply at this time. By far the most abundant invertebrates in oak, during the breeding season, were Lepidopteran larvae. Although scarce prior to the onset of laying, by around the time of mean onset of laying of the population, numbers were beginning to increase quite rapidly. In terms of biomass, it can be seen that available caterpillar tissue increased dramatically and rapidly through the laying period. Therefore, any nutritional constraints on early laying birds, imposed by limited caterpillar availability, would probably quickly disappear. Although oak is generally considered to have the highest species richness of all trees in Britain (Southwood, Moran & Kennedy 1982), most other invertebrates remained at fairly low levels during the period of sampling, with Araneae being the most abundant of these, followed by Coleoptera. Very few invertebrates were found in birch, although three relatively large larvae were found in catkins and blue tits were observed tearing at birch catkins.

In a similar study conducted by Woodburn (1997), in the mixed woodland of Wytham Wood, near Oxford, arthropod abundance was measured in five different species of deciduous tree at two times in the breeding season. During the prelaying period, oak exhibited the lowest

abundance and biomass of arthropods of the five tree species considered; the greatest abundance and biomass of invertebrates at this time occurring in hawthorn and hazel, due predominantly to high levels of Coleoptera. However, by the time most birds were laying, oak had the greatest abundance and biomass of invertebrates of any tree species examined. When the foraging locations of blue tits were investigated, it was found that although over the whole season they showed a significant preference for oak, in relation to its abundance, a considerable proportion of foraging still occurred in other tree species, in particular hawthorn and hazel. Although no data are given on changes in foraging location through the season, hawthorn and hazel do come into leaf significantly earlier than oak (rise in arthropod abundance, in particular phytophagous arthropods, is closely related to bud burst) and it was reported that birds used tree species largely in accordance with their arthropod abundance at the time. Correspondingly, in an examination by Woodburn (1997) of the gizzard contents of two laying blue tits, Coleoptera accounted for a greater proportion of the gizzard contents than any other arthropod order; followed by Lepidoptera and Araneae. Other studies conducted in mixed woods have also found that, although there is a preference for oak, a considerable proportion of foraging (particularly early in the season) is also conducted in other tree species (Gibb 1954; Hartley 1953).

In contrast to these studies, Betts' (1955) investigation of Parid diets was conducted within an oak plantation. In this situation, blue tits ability to switch tree species in order to track arthropod abundance was restricted and, thus, when preferred arthropods were not readily available in oak, tits had to feed on other food items. Betts found that many birds' gizzards contained a large proportion of oak bud tissue at this time (all birds' gizzards contained over 40% bud tissue, and many had over 75%;compared with Woodburn's study where the mean at this time was 9%). It is difficult to distinguish whether bud tissue is taken unintentionally whilst searching for eggs, larvae and pupae, or eaten deliberately. Plant material is generally of lower nutritional quality than animal tissue; in particular it is often considerably lower in the amino acids methionine, cysteine and lysine. In addition, plants can only partially be digested by species which do not have digestive tracts adapted for herbivory, because all higher vertebrates lack an enzyme system which can digest the cellulose cell wall of plants (Schmidt-Nielsen 1990). Therefore, blue tits breeding in woodland which is heavily dominated by oak (such as our study site) may face greater potential nutritional limitations on egg production early in the breeding season, as a result of both lower arthropod food availability and increased proportion of lower quality plant food in the diet, than birds breeding in more mixed woods. Later in the breeding season, however, birds breeding in oak dominated woods may have access to greater food resources than birds breeding in mixed woods.

## 2.5 Amino acid composition of blue tit eggs and prey items

### 2.5.1 Methods

#### 2.5.1.1 Arthropod killing methods

I used two methods because my original technique using ethyl acetate proved impractical for large samples.

Ethyl Acetate:

Straight sided glass vials (12.8mm x 50mm) with corked tops were used for this technique. In the laboratory, prior to embarking upon a days collection, around 6 vials were filled with cotton wool. Approximately 0.5ml of ethyl acetate was then added to the cotton wool in each vial and the cork stopper replaced firmly. These vials, together with several empty, corked vials were then taken into the field. Collected arthropods were placed in an empty vial. When they were to be killed, the cork stopper from the vial containing the arthropods was exchanged with a cork from one of the vials containing ethyl acetate and replaced firmly. Within a few minutes, the vapour from the cork had killed all the arthropods in the vial. Collected arthropods could then either be left in the vial until the collector returned to the laboratory or transferred to another container.

Chloroform:

In the laboratory, prior to commencing collection, several drops of chloroform were placed inside a kilner jar lined with porous plaster and the lid replaced firmly. In the field, arthropods to be killed using this technique were collected in small glass jars. When a sample of arthropods was to be killed, the lid was removed from the collecting jar and a loose plug of cotton wool inserted to prevent any arthropods climbing out. The collecting jar was then placed inside the kilner jar and the lid replaced. Within a few minutes all the arthropods would be dead. The collecting jar(s) could then be removed and either the lids replaced and the sample returned to the lab like this, or the contents transferred to another container.

#### 2.5.1.2 Sample collection

All the invertebrates found whilst examining twigs in the course of the abundance survey (section 2.3) were collected. These arthropods were killed using Ethyl Acetate, weighed, placed in labelled and sealed glass vials and stored at -20°C. This did not, generally, provide enough material for nutritional analysis, however, and additional invertebrates had to be collected by

using beating trays. It was found that conventional beating techniques (a hand held beating tray of approx. $1m^2$ and a short stick) were inadequate due to the height of the 'lower' branches and the low density of invertebrates early in the season. Therefore, a technique on a somewhat larger scale was developed. Two large cloth sheets (approx. 3m x 2m each) were laid out on the ground beneath the chosen tree and the branches above struck hard several times using a 6m long length of timber (in actual fact, a slender debranched tree trunk). Any invertebrates which fell onto the sheets were then collected and placed in labelled glass vials according to order, or in some cases family. If only a small number of arthropods fell onto the sheet, they were all collected before being killed. If, however, there were many, collected arthropods were killed at approximately 5 minute intervals. All arthropods, except Lepidopteran larvae were killed using Ethyl Acetate. Small numbers of caterpillars were also killed using Ethyl Acetate, but Chloroform was found to be more convenient for killing larger numbers of larger caterpillars. After collection and killing, arthropods were taken back to the laboratory, placed in labelled vials, weighed and frozen at -20°C. All samples were frozen within 2 hours of collection.

Blue tit eggs were collected (under licence from SNH) from clutches laid around the middle of the population laying period. Two eggs were collected from each of 5 clutches.

#### 2.5.1.3 Sample preparation

Frozen prey samples were freeze dried for 5 days and then re-weighed. Samples were then ground, using a mortar and pestle, divided into subsamples and transferred into labelled vials. These were stored at -20°C until analysed.

The overall nutritional quality of caterpillars may be significantly influenced by the contents of the gut, if the gut contains a relatively large volume of leaf material. Therefore, in order to investigate this, 20 relatively large caterpillars, collected in late June, were dissected and their gut contents removed. The gut contents and caterpillar bodies minus gut contents were then prepared for analysis as above.

To solidify contents and facilitate separation into shell, yolk and albumen, blue tit eggs were cooked in an oven at 100°C for 1 hour; having first made a small hole at the airspace to allow expanding gas to escape. Separated constituents were weighed, freeze dried for 5 days, and then weighed again.

#### 2.5.1.4 Amino acid analysis

Complete amino acid profile analyses were carried out by Experiment Station Chemical Laboratories, at the University of Missouri. The procedures used were those recommended by the Association of Official Analytical Chemists (A.O.A.C.) in AOAC Methods 15: 982.30 E(a,b,c), 1990. Two replicates were performed for each material under investigation. For blue tit eggs, each replicate was an individual egg from a different clutch; whilst for all the invertebrates, each replicate was a subsample from an homogenised sample of many individuals.

## 2.5.2 Results

### 2.5.2.1 Amino acid analyses

Amino acids are grouped into '*Essential*', '*Semi-essential*' and '*Non-essential*' amino acids. Essential amino acids (EAA) cannot be synthesised by the animal and must, therefore, be ingested in the diet. Non-essential amino acids (NEAA) are those which can be produced adequately from non-specific precursors within the animal. Semi-essential amino acids (SEAA) are those, such as cysteine and tyrosine, which can be derived metabolically, but only from specific essential amino acids; methionine and phenylalanine, respectively, in this case. If SEAA are present in the diet they can have a sparing effect on the animal's requirements for the respective EAA. Maintenance requirements for methionine and phenylalanine are usually determined in the absence of dietary cysteine and tyrosine. If cysteine and tyrosine are present in the diet, they can contribute up to 50% of determined maintenance requirements for methionine and phenylalanine, respectively.

In the results below, figures with the suffix 'a' present amino acid composition data in terms of g/100g dry weight, whilst those with the suffix 'b' present it in terms of g/100g protein. This allows one to compare different materials in terms of protein quality rather than just absolute amino acid quantity. The mean amino acid compositions of blue tit egg yolk and albumen are presented in Figure 2.6a and 2.6b The mean composition of whole blue tit eggs has been calculated from these data and is presented in Figure 2.7 along with amino acid composition data from the literature for eggs of other species. Figures 2.8a. and 2.8b present mean amino acid composition data for whole blue tit eggs and all the potential prey items investigated. The mean amino acid composition of the gut contents of late season caterpillars and bodies minus gut contents are presented in Figures 2.9a and 2.9b and, as above, the mean composition of whole late season caterpillars has been calculated from these data and presented in the same figures. The mean amino acid compositions (in g/100g dry weight), of all materials investigated, are also presented in Table 2.2, primarily for the reference of future investigators. The total protein content of all samples is presented in Table 2.3.

**Table 2.3:** Mean amino acid compositions of blue tit eggs and prey items (g/100g dry weight; mean ± S.E.)

| Amino acids | Blue tit Albumen | Blue tit Yolk | Whole blue tit egg (yolk and albumen combined) | 26th May - Caterpillars | 14th June - Caterpillars | Late season caterpillars' gut contents | Late season caterpillars minus their gut contents | Late season caterpillars (guts and bodies combined) | Wolf spiders | Small spiders |
|---|---|---|---|---|---|---|---|---|---|---|
| Taurine | 0.055 ± 0.025 | 0.045 ± 0.025 | 0.049 ± 0.025 | 0.015 ± 0.005 | 0.020 ± 0.000 | 0.030 ± 0.010 | 0.020 ± 0.000 | 0.022 ± 0.002 | 2.135 ± 0.055 | 0.720 ± 0.030 |
| Hydroxyproline | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.055 ± 0.015 | 0.050 ± 0.000 | 0.185 ± 0.015 | 0.010 ± 0.010 | 0.044 ± 0.011 | 0.035 ± 0.035 | 0.045 ± 0.005 |
| Aspartic Acid | 6.535 ± 0.005 | 2.270 ± 0.000 | 3.857 ± 0.112 | 4.215 ± 0.325 | 3.925 ± 0.035 | 1.630 ± 0.050 | 4.050 ± 0.320 | 3.575 ± 0.267 | 5.730 ± 0.080 | 5.340 ± 0.160 |
| Threonine | 3.465 ± 0.025 | 1.205 ± 0.005 | 2.045 ± 0.052 | 2.195 ± 0.145 | 2.005 ± 0.015 | 0.845 ± 0.005 | 1.915 ± 0.055 | 1.705 ± 0.045 | 2.440 ± 0.110 | 2.420 ± 0.070 |
| Serine | 4.005 ± 0.195 | 2.055 ± 0.005 | 2.775 ± 0.019 | 2.195 ± 0.265 | 1.865 ± 0.045 | 0.745 ± 0.025 | 1.635 ± 0.005 | 1.460 ± 0.009 | 2.175 ± 0.145 | 2.930 ± 0.130 |
| Glutamic Acid | 9.205 ± 0.035 | 2.585 ± 0.065 | 5.048 ± 0.117 | 6.735 ± 0.905 | 5.105 ± 0.095 | 2.300 ± 0.130 | 5.340 ± 0.570 | 4.743 ± 0.484 | 7.670 ± 0.050 | 7.215 ± 0.205 |
| Proline | 2.910 ± 0.040 | 0.875 ± 0.005 | 1.633 ± 0.071 | 2.230 ± 0.150 | 2.195 ± 0.055 | 0.970 ± 0.120 | 2.290 ± 0.230 | 2.031 ± 0.208 | 2.355 ± 0.005 | 2.380 ± 0.070 |
| Lanthionine | 0.125 ± 0.025 | 0.280 ± 0.020 | 0.224 ± 0.007 | 0.085 ± 0.045 | 0.145 ± 0.025 | 0.085 ± 0.015 | 0.095 ± 0.005 | 0.093 ± 0.007 | 0.030 ± 0.030 | 0.035 ± 0.005 |
| Glycine | 2.365 ± 0.075 | 0.740 ± 0.020 | 1.347 ± 0.057 | 2.750 ± 0.330 | 2.255 ± 0.075 | 1.035 ± 0.065 | 2.515 ± 0.355 | 2.224 ± 0.298 | 3.360 ± 0.010 | 3.355 ± 0.215 |
| Alanine | 2.870 ± 0.050 | 1.300 ± 0.000 | 1.885 ± 0.059 | 3.190 ± 0.230 | 3.025 ± 0.005 | 1.040 ± 0.020 | 2.805 ± 0.205 | 2.458 ± 0.169 | 3.805 ± 0.065 | 3.550 ± 0.200 |
| Cysteine | 2.085 ± 0.055 | 0.430 ± 0.010 | 1.037 ± 0.015 | 0.675 ± 0.065 | 0.570 ± 0.020 | 0.325 ± 0.015 | 0.520 ± 0.020 | 0.482 ± 0.019 | 0.940 ± 0.040 | 0.930 ± 0.040 |
| Valine | 4.715 ± 0.015 | 1.630 ± 0.040 | 2.777 ± 0.110 | 2.755 ± 0.145 | 2.565 ± 0.025 | 1.080 ± 0.050 | 2.765 ± 0.265 | 2.434 ± 0.223 | 3.395 ± 0.035 | 3.045 ± 0.095 |

**Table 2.3cont:** Mean amino acid compositions of blue tit eggs and prey items (g/100g dry weight; mean ± S.E.)

| Amino acids | Blue tit Albumen | Blue tit Yolk | Whole blue tit egg (yolk and albumen combined) | 26th May - Caterpillars | 14th June - Caterpillars | Late season caterpillars' gut contents | Late season caterpillars minus their gut contents | Late season caterpillars (guts and bodies combined) | Wolf spiders | Small spiders |
|---|---|---|---|---|---|---|---|---|---|---|
| Methionine | 1.210 ± 0.010 | 0.470 ± 0.010 | 0.745 ± 0.009 | 1.045 ± 0.065 | 0.865 ± 0.005 | 0.335 ± 0.015 | 0.845 ± 0.065 | 0.745 ± 0.055 | 1.435 ± 0.015 | 1.295 ± 0.035 |
| Isoleucine | 4.620 ± 0.140 | 1.620 ± 0.030 | 2.732 ± 0.044 | 2.210 ± 0.150 | 2.030 ± 0.030 | 0.880 ± 0.060 | 2.275 ± 0.215 | 2.001 ± 0.185 | 2.905 ± 0.045 | 2.780 ± 0.110 |
| Leucine | 6.190 ± 0.090 | 2.335 ± 0.025 | 3.766 ± 0.0825 | 3.750 ± 0.280 | 3.315 ± 0.035 | 1.310 ± 0.030 | 3.420 ± 0.150 | 3.006 ± 0.126 | 4.820 ± 0.160 | 4.520 ± 0.210 |
| Tyrosine | 3.225 ± 0.035 | 1.280 ± 0.010 | 2.002 ± 0.043 | 2.180 ± 0.240 | 2.385 ± 0.025 | 0.855 ± 0.005 | 2.965 ± 0.065 | 2.551 ± 0.053 | 3.225 ± 0.055 | 2.695 ± 0.375 |
| Phenylalanine | 3.475 ± 0.075 | 1.165 ± 0.035 | 2.025 ± 0.109 | 2.035 ± 0.115 | 1.845 ± 0.025 | 0.860 ± 0.020 | 2.025 ± 0.155 | 1.796 ± 0.128 | 2.555 ± 0.005 | 2.385 ± 0.075 |
| Hydroxylysine | 0.000 ± 0.000 | 0.125 ± 0.015 | 0.078 ± 0.006 | 0.060 ± 0.030 | 0.045 ± 0.005 | 0.005 ± 0.005 | 0.070 ± 0.010 | 0.057 ± 0.009 | 0.090 ± 0.010 | 0.015 ± 0.005 |
| Histidine | 1.595 ± 0.025 | 0.665 ± 0.025 | 1.011 ± 0.049 | 1.580 ± 0.100 | 1.485 ± 0.015 | 0.735 ± 0.025 | 1.520 ± 0.130 | 1.366 ± 0.109 | 1.810 ± 0.010 | 1.715 ± 0.045 |
| Ornithine | 0.055 ± 0.005 | 0.055 ± 0.005 | 0.055 ± 0.001 | 0.090 ± 0.020 | 0.055 ± 0.005 | 0.060 ± 0.010 | 0.055 ± 0.005 | 0.056 ± 0.006 | 0.040 ± 0.010 | 0.020 ± 0.000 |
| Lysine | 5.450 ± 0.050 | 2.340 ± 0.090 | 3.493 ± 0.118 | 3.865 ± 0.235 | 3.080 ± 0.010 | 1.230 ± 0.020 | 3.300 ± 0.270 | 2.894 ± 0.221 | 4.435 ± 0.055 | 4.125 ± 0.135 |
| Arginine | 3.260 ± 0.050 | 1.755 ± 0.045 | 2.312 ± 0.048 | 3.490 ± 0.250 | 2.600 ± 0.050 | 1.215 ± 0.025 | 2.870 ± 0.175 | 2.541 ± 0.146 | 4.800 ± 0.110 | 3.725 ± 0.165 |
| Tryptophan | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 |

**Table 2.4**: Mean protein content (± SE) of blue tit eggs and potential invertebrate prey.

| Samples | Mean protein content (% of dry weight) ± SE |
|---|---|
| blue tit albumen | 67.4 ± 0.4 |
| blue tit yolk | 25.2 ± 0.2 |
| Whole blue tit egg | 40.9 ± 1.1 |
| Wolf spiders | 60.2 ± 0.9 |
| Small arboreal spiders | 55.2 ± 2.4 |
| 26$^{th}$ May caterpillars | 47.4 ± 3.9 |
| 14$^{th}$ June caterpillars | 41.4 ± 0.2 |
| Late season caterpillar gut contents | 17.8 ± 0.6 |
| Late season caterpillars minus gut contents | 43.3 ± 3.2 |
| Whole late season caterpillars | 38.3 ± 2.7 |

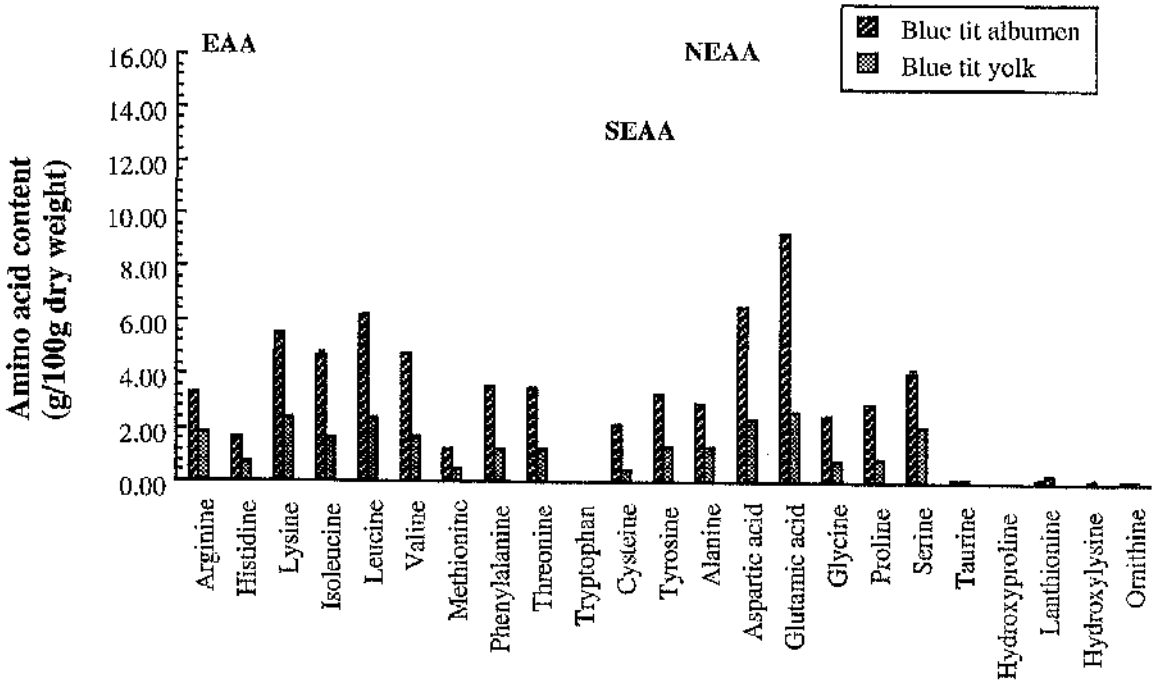



**Figure 2.6a**: Mean (+ SE, *n*=2) amino acid composition of albumen and yolk in egg of the blue tit (expressed as g/100g dry weight)

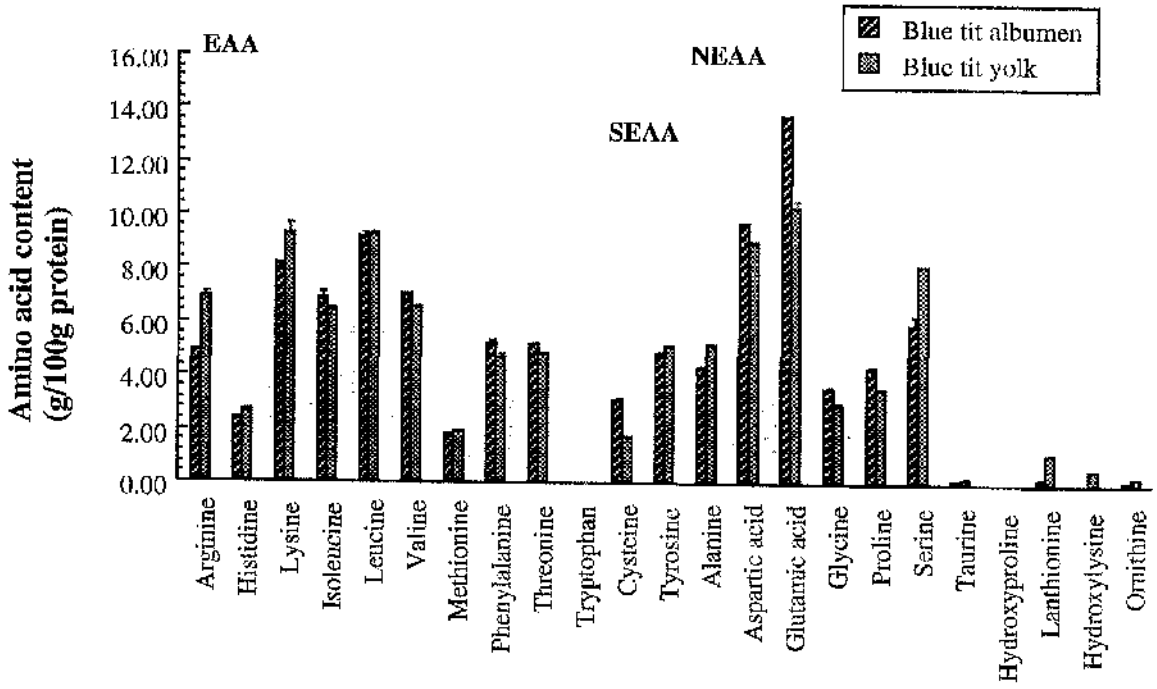



**Figure 2.6b**: Mean (+ SE, *n*=2) amino acid composition of albumen and yolk in egg of the blue tit (expressed as g/100g total protein)

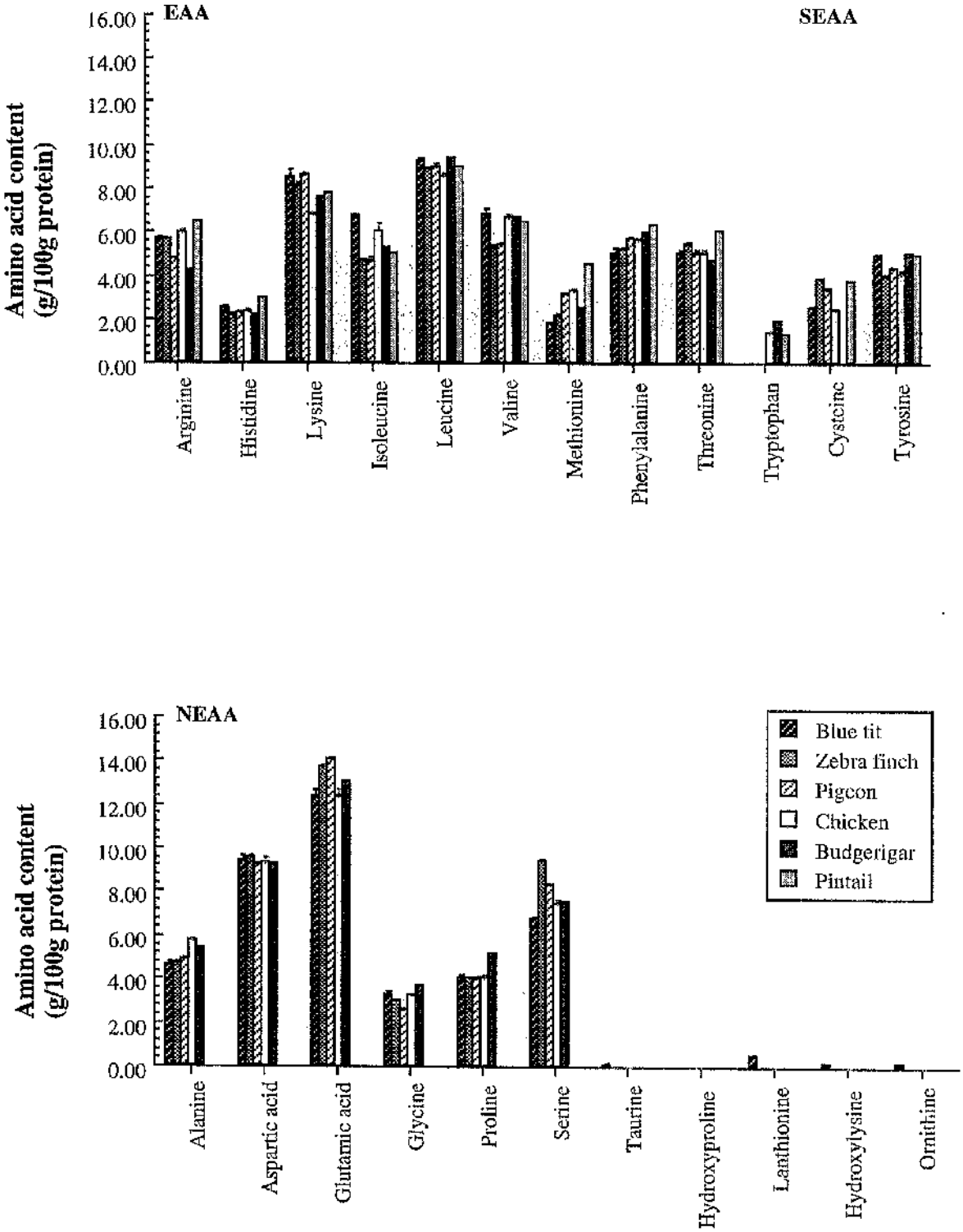



**Figure 2.7**: Mean (+ SE, *n*=2) amino acid composition (expressed as g/100g protein) of blue tit eggs and eggs from five other avian species (data from Murphy 1994)

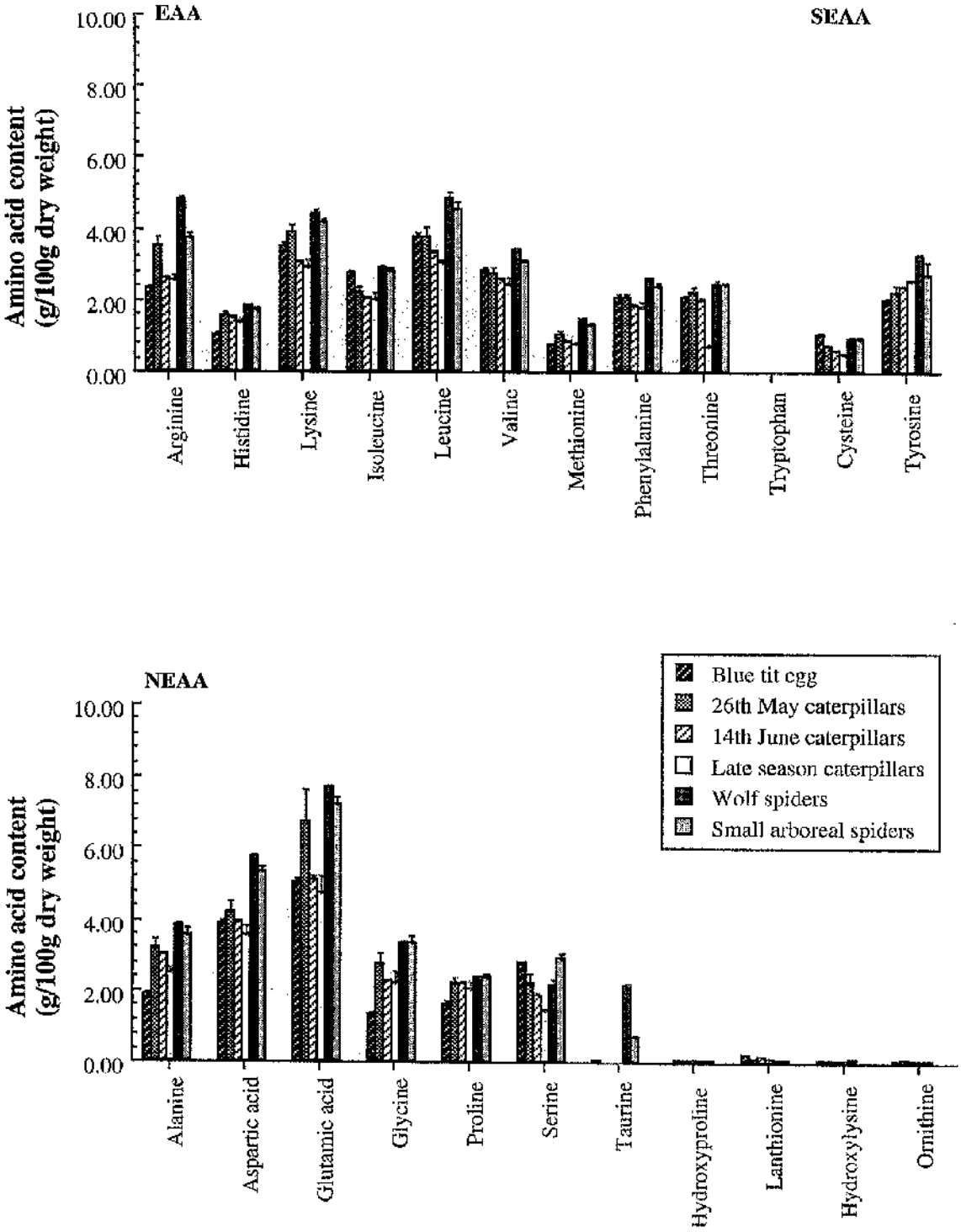



**Figure 2.8a**: Mean (+ SE, *n*=2) amino acid compositions of blue tit eggs and selected potential prey items (expressed as g/100g dry weight)

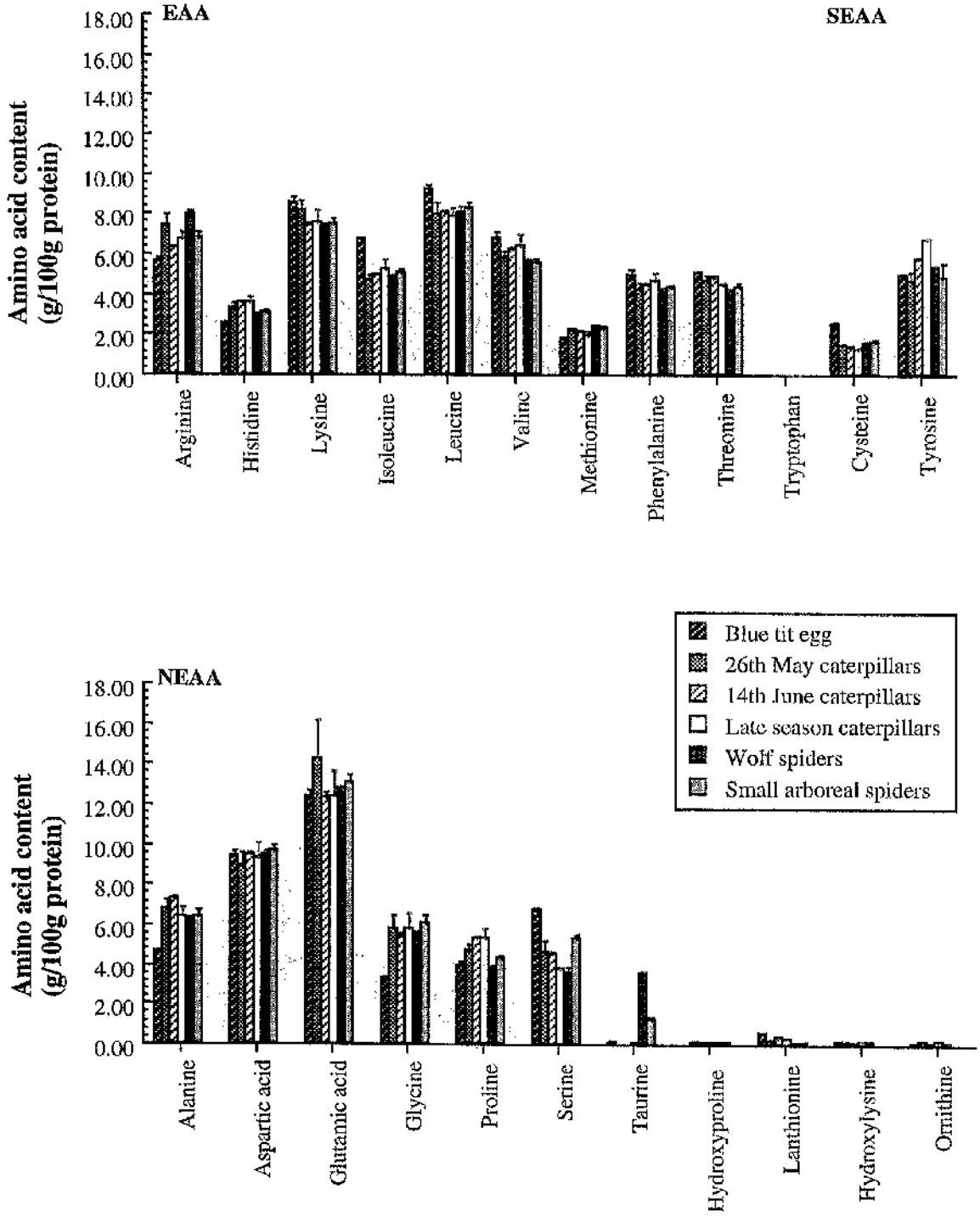



**Figure 2.8b**: Mean (+ SE, *n*=2) amino acid composition of blue tit eggs and selected potential prey items (expressed as g/100g total protein)

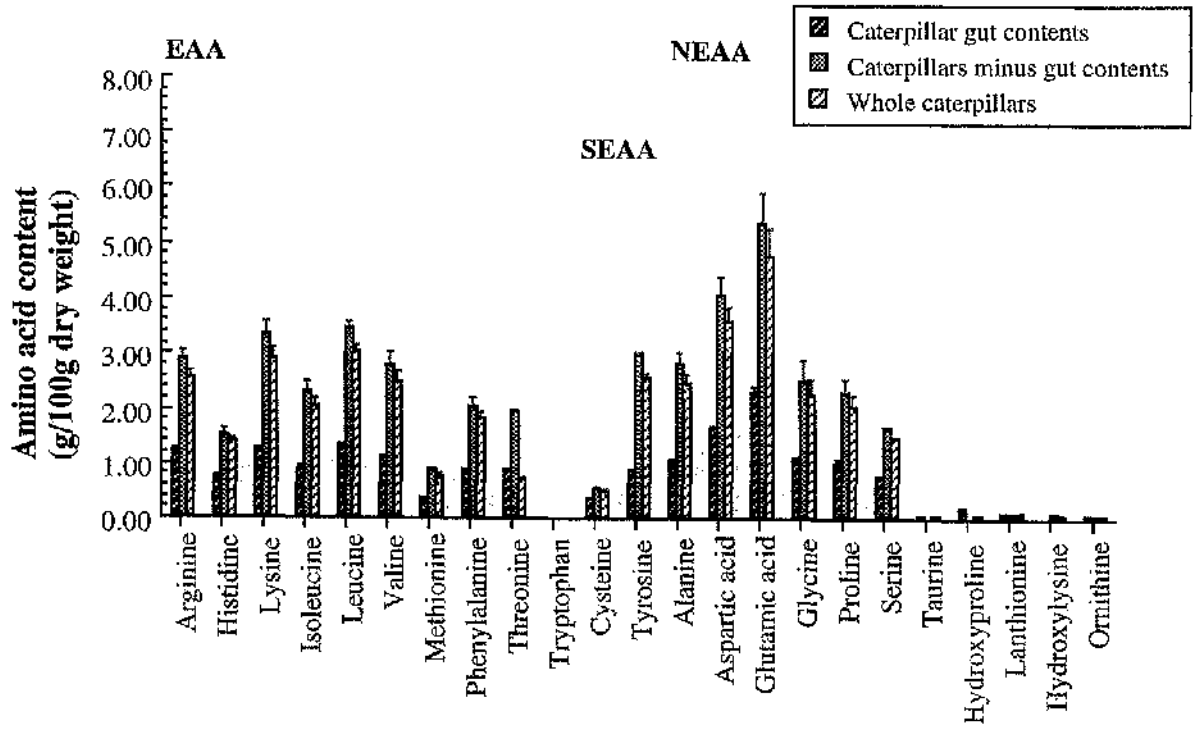



**Figure 2.9a**: Mean (+ SE, *n*=2) amino acid composition of late season caterpillars, their gut contents, and bodies minus gut contents (expressed as g/100g dry weight)

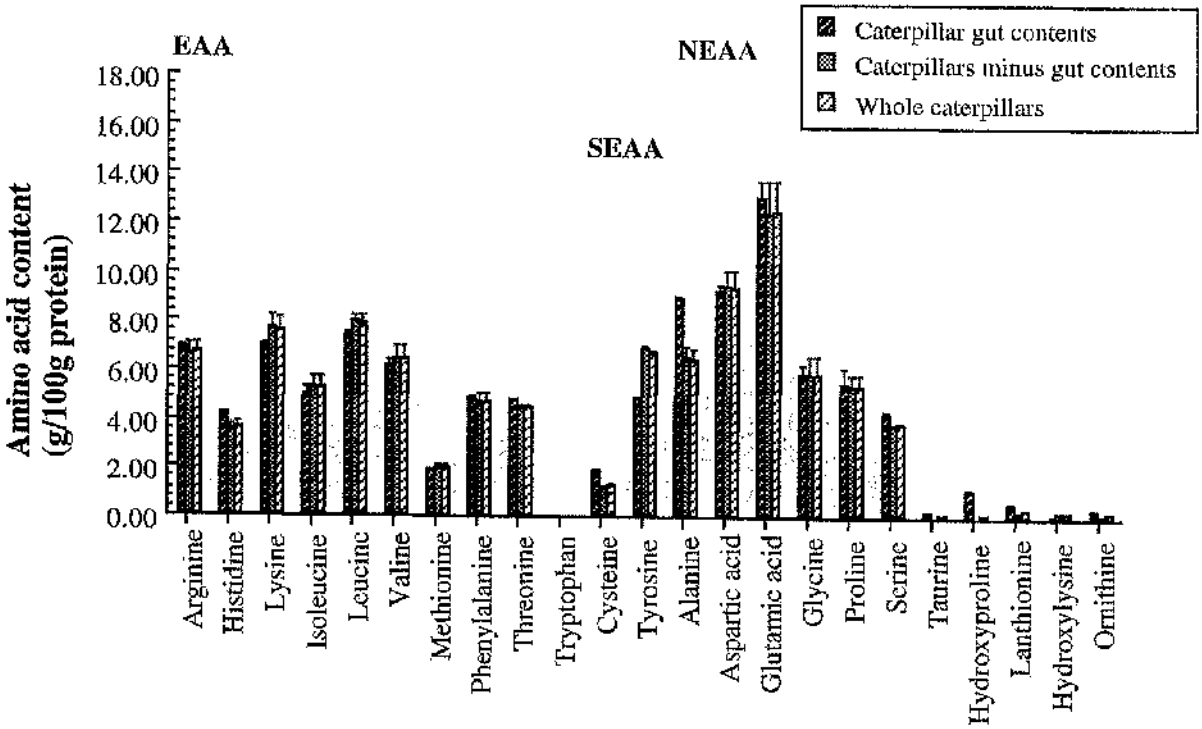



**Figure 2.9b**: Mean (+ SE, *n*=2) amino acid composition of late season caterpillars, their gut contents, and bodies minus gut contents (expressed as g/100g total protein)

### 2.5.2.2 Estimated daily amino acid requirements of laying blue tits

A. MAINTENANCE

Murphy (1993) determined the daily EAA requirements for maintenance of captive white-crowned sparrows, in terms of mg/kJ basal energy expenditure. Since maintenance requirements amongst small passerines are probably fairly consistent, these data were used to calculate EAA maintenance requirements for blue tits.

Firstly, the equations of Aschoff & Pohl (1970b) for BMR in passerines, were used to calculate an estimate of BMR for blue tits. These equations differ between *active phase* and *non-active phase*:

Active phase BMR (Kcal/day) $= 140.9 \times \text{mass(Kg)}^{0.704}$

Non-active phase BMR (Kcal/day) $= 114.8 \times \text{mass(Kg)}^{0.726}$

The average mass of a female blue tit during laying (including reproductive tissue, but excluding any fully formed eggs) is about 12.0g (Perrins 1979). Thus:

Active phase BMR (Kcal/day) $= 140.9 \times (0.012)^{0.704}$

$= 6.26$ Kcal/day

Non-active phase BMR (Kcal/day) $= 114.8 \times (0.012)^{0.726}$

$= 4.63$Kcal/day

If birds are active for approximately 16 hours per day, at this time of year, then

average BMR = 5.72Kcal/day (= 23.93kJ/day).

Estimated EAA maintenance requirements of a 12g blue tit are presented in Table 2.4a.

Values for methionine and phenylalanine are in the absence of cysteine and tyrosine respectively. Cysteine and tyrosine could supply about half of the below requirements for methionine and phenylalanine, respectively (Murphy 1993).

**Table 2.5a**: Estimated daily EAA maintenance requirement of a laying blue tit

| Amino acid | Maintenance requirement (μmoles/day) |
|---|---|
| Arginine | 59.75 |
| Histidine | 19.19 |
| Lysine | 54.14 |
| Isoleucine | 57.10 |
| Leucine | 80.36 |
| Valine | 57.93 |
| Methionine | 69.31 |
| Phenylalanine | 63.41 |
| Threonine | 44.97 |
| Tryptophan | < 8.93 |

B. EGG PRODUCTION

The mean fresh egg weight for blue tits in our study population was 1.138g and the mean fresh weight of constituents: 0.083g shell, 0.262g yolk and 0.793g albumen. The mean dry masses of these components were: 0.070g, 0.125g and 0.086g. Using the amino acid analysis of eggs data from Table 2.2, the mean amino acid composition of blue tit eggs in our study population was calculated and is presented in Table 2.4b. Assuming a utilisation efficiency of 75% (Fisher 1980: Scott *et al* 1982), the quantity of amino acids required in the diet for the formation of one egg was calculated and is also presented in Table 2.4b. Required dietary quantities of methionine and phenylalanine are in the absence of cysteine and tyrosine, respectively. In egg protein, cysteine accounts for 64% of total SAA and tyrosine accounts for 47% of total combined phenylalanine and tyrosine. Consequently, dietary cysteine could probably substitute for 64% of quoted requirements for dietary methionine and tyrosine could probably substitute for 47% of quoted requirements for dietary phenylalanine.

**Table 2.5b**: Estimated EAA content of one blue tit egg, and estimated dietary requirement assuming a 75% utilisation efficiency.

| EAA & SEAA | Quantity in whole blue tit egg (μmoles/egg) | Quantity required in diet for formation of one egg (μmoles/egg) |
|---|---|---|
| Arginine | 32.00 | 42.66 |
| Histidine | 16.06 | 21.42 |
| Lysine | 59.39 | 79.18 |
| Isoleucine | 53.01 | 70.68 |
| Leucine | 72.84 | 97.12 |
| Valine | 61.46 | 81.94 |
| Methionine | 12.41 | 46.45 |
| Phenylalanine | 30.20 | 76.00 |
| Threonine | 44.37 | 59.16 |
| Tryptophan | ≤ 0.54 | ≤ 0.72 |
| Cysteine | 22.43 | |
| Tyrosine | 26.80 | |

**2.5.2.3 Estimated daily amino acid requirements in relation to dietary supply.**

King (1973) showed that, in birds where a succession of follicles begin development at daily intervals, the daily metabolic output required for the formation of the clutch reaches a peak that is equivalent to the total energy expenditure on a single egg. This peak value is reached $p$ -1 days after the onset of rapid yolk deposition in the first follicle , where $p$ = the number of days over which a follicle grows. This peak investment in egg production must be sustained for as long as new follicles begin enlargement each day. Egg formation in tits takes about 4 days (Kluijver 1951) and mean clutch size for blue tits is around 10 - 11 eggs. Consequently, this peak level of investment must often be sustained for over 1 week. King's model can also be applied to protein requirements and, therefore, peak level of protein investment in egg production is equivalent to the total protein investment in a single egg.

Estimated total daily EAA requirements for maintenance and egg production are thus obtained by summing the values from Tables 2.4a and 2.4b, and are presented in Table 2.4c.

By combining the values given above, for the proportion of estimated methionine and phenylalanine requirements, for egg production and maintenance, that can be substituted with cysteine and tyrosine, respectively, dietary cysteine and tyrosine could substitute for about 56% and 48% of total estimated dietary requirements for methionine and phenylalanine, respectively. The EAA and SEAA contents of small arboreal spiders and caterpillars from around 26$^{th}$ May are also presented in Table 2.4c Values for tryptophan have been omitted because they fell below accurate detectable levels in analyses.

**Table 2.5c:** Total daily EAA and SEAA requirements for a laying blue tit and content in small spiders and caterpillars

| EAA & SEAA | Total dietary requirement (μmoles/day) | EAA & SEAA content of 26th May caterpillars (μmoles/g dry mass) | EAA & SEAA content of small arboreal spiders (μmoles/g dry mass) |
|---|---|---|---|
| Arginine | 102.41 | 223.45 | 238.49 |
| Histidine | 40.612 | 115.21 | 125.05 |
| Lysine | 133.33 | 301.54 | 321.83 |
| Isoleucine | 127.77 | 195.30 | 245.67 |
| Leucine | 177.47 | 331.39 | 399.43 |
| Valine | 139.88 | 277.91 | 307.16 |
| Methionine | 115.77 | 79.65 | 98.71 |
| Phenylalanine | 139.41 | 138.27 | 162.05 |
| Threonine | 104.13 | 217.10 | 239.35 |
| Tryptophan | | | |
| Cysteine | | 65.45 | 90.17 |
| Tyrosine | | 133.60 | 165.16 |

The total dry masses of small spiders or small caterpillars that would need to be ingested to satisfy estimated daily requirements for each amino acid, are presented in Table 2.4d. The values for methionine & cysteine and phenylalanine & tyrosine were calculated with the assumption that dietary cysteine and tyrosine could provide a maximum of 56% and 48%, respectively, of the requirement for methionine & cysteine and phenylalanine & tyrosine, respectively (see above).

From Table 2.4d, it can be seen that sulphur-containing amino acids (SAA) would be the most limiting dietary amino acids.

**Table 2.5d**: Estimated mass of caterpillars or small spiders required to satisfy daily requirement for each amino acid (mg dry mass/day)

| EAA & SEAA | Estimated mass of 26th May caterpillars required to satisfy dietary requirement (mg dry mass/day) | Estimated mass of small arboreal spiders required to satisfy dietary requirement (mg dry mass/day) |
|---|---|---|
| Arginine | 458 | 429 |
| Histidine | 353 | 325 |
| Lysine | 442 | 414 |
| Isoleucine | 654 | 520 |
| Leucine | 536 | 444 |
| Valine | 503 | 455 |
| Threonine | 480 | 435 |
| Tryptophan | | |
| Methionine & Cysteine | 798 | 613 |
| Phenylalanine & Tyrosine | 524 | 447 |

## 2.6 Discussion II

BLUE TIT EGGS

Figure 2.7 presents data on the amino acid composition of whole blue tit eggs plus data from the literature for five other avian species. The levels for blue tits are very similar to those for other species, although there are a few noteworthy differences. Levels of phenylalanine are slightly lower than for other species, but this is more or less balanced by relatively higher levels of tyrosine. The most striking difference is in the sulphur amino acids (SAA). Blue tit eggs have the lowest levels of methionine of all the species for which data are available, the next lowest being for zebra finches. Turning to cysteine, we see that in species other than blue tits, low methionine levels are generally counteracted by higher cysteine levels. For example, the eggs of zebra finches, pigeons and chickens have, in that sequence, increasing proportions of methionine (2.19, 3.07 & 3.25 g/100g protein) and decreasing proportions of cysteine (3.82, 3.34 & 2.25 g/100g protein). As a result, although methionine and cysteine levels vary quite considerably between species (up to 48% and 70% respectively), the combined levels of methionine and cysteine (6.01, 6.41 & 5.5 g/100g protein respectively) are more similar (maximum of 17% difference). In blue tit eggs, however, cysteine levels are the second lowest of all the species for which data were available; being only fractionally greater than that for chickens. As a result, combined methionine and cysteine levels are only 4.36 g/100g protein.

Further, levels of tryptophan (the only other sulphur-containing essential amino acid) are considerably lower than those in other species where it has been measured. Levels for chickens, budgerigars and pintails were 1.41, 1.88 and 1.28 g/100g protein, respectively, whilst in blue tits it was below 0.1 g/100g protein. In a study by Donnan (1993), which examined the amino acid composition of zebra finch eggs, tryptophan was, similarly, detected only in trace levels. However, the levels of several other amino acids were unusually high or low in Donnan's study compared with published results for other species and Murphy's (1994) results for Zebra finches. It is possible, therefore, that the results from Donnan's study were somewhat aberrant and it would perhaps be wise to treat his result for tryptophan with some caution. Thus, levels of tryptophan may vary quite considerably between species, with blue tits not being exceptional. However, in conjunction with low methionine and cysteine levels, this result does compound the finding that the SAA content of blue tit eggs is comparatively low. Why, then, should blue tit eggs contain somewhat low levels of SAA?

Firstly, it is possible that the blue tit eggs examined were unusual or aberrant in some way and thus unrepresentative. Although this is possible, since only two eggs were analysed, it

is very unlikely. Levels of all other amino acids were very similar to those found in other avian species; total proportional protein contents of yolk and albumen (Table 2.3) were similar to those reported for great tits (Yoo, 1993); and each egg came from a different clutch, yet the standard errors for mean levels of cysteine and methionine are extremely small for both yolk and albumen - in fact, amongst the smallest standard errors for any of the amino acids examined. If the eggs were in any way aberrant, one would not expect two independent eggs, from different clutches, to be so similar. Further, although only 2 eggs were analysed, four separate analyses were carried out since yolk and albumen were analysed separately. The pattern of methionine and cysteine levels in yolk and albumen (Figure 2.6a,b) was very similar to that seen in Murphy's (1994) zebra finches; methionine levels virtually identical in yolk and albumen, and levels of cysteine higher in albumen than yolk.

A second possible explanation is that female blue tits are unable to obtain enough SAA in the diet to maintain optimum levels in the eggs, i.e. they face a nutritional constraint. Again, however, this is very unlikely. If such a constraint were to exist, it would be most likely to occur with those birds laying particularly early in the season. At this time, invertebrates are still relatively scarce and there are reports of blue tits' gizzards containing quite high proportions of oak bud tissue; possibly ingested incidentally whilst foraging for bud-inhabiting larvae. Plant tissue, such as this, is generally a relatively poor supply of SAA (Scott, Nesheim & Young 1982), and in herbivorous or granivorous birds, SAA and lysine are generally reported to be the first limiting amino acids during egg production (Houston *et al* 1995a; Murphy 1994; Murphy & King 1992; Scott, Nesheim & Young 1982). The eggs analysed in this study, however, were collected from birds laying mid season, when females should have had better access to invertebrates and, further, the blue tit eggs analysed had the highest levels of lysine of any of the avian species for which data were available (Figure 2.7). In addition, birds such as zebra finches and pigeons, which feed primarily on a plant-derived diet, appear to produce eggs which contain significantly higher levels of SAA.

A third hypothesis, is that blue tit eggs do not have higher levels of SAA because they simply do not need them; because developing, pre-hatching young do not require as much as those of other species and/or because the young can get a good supply of SAA after hatching, from the invertebrate prey supplied by their parents. Blue tits are altricial and, therefore, may require less SAA in the egg for feather production than a precocial species. However, zebra finches, pigeons and budgerigars are also altricial but have significantly higher levels of SAA than blue tits. Further, although the precocial pintail duck has considerably higher levels of SAA than the other species, the chicken, another precocial species, has lower combined levels of methionine and cysteine than both zebra finches and pigeons.

Once hatched, blue tit young receive an invertebrate diet which is relatively rich in protein and contains protein of higher quality than plant material. Perhaps other altricial species, whose young receive a diet containing a lower quantity/quality of protein, require more SAA in the egg to help them form down feathers and satisfy SAA requirements in the first day or two following hatching. Data from more species, particularly those whose young receive a high quality diet, however, would be required to substantiate this hypothesis.

INVERTEBRATES

Spiders have considerably higher protein contents than caterpillars (55-60% versus 38-47%), with the protein content of wolf spiders being about 5% greater than that for assorted small, arboreal spiders. Within caterpillars, protein content fell from 47% to 38% of dry body weight as they developed and grew through the season (although the actual mass of protein contained per caterpillar increases throught the season as a result of their increase in size). This may reflect a change in actual body composition or perhaps simply a greater proportion of gut contents in larger caterpillars. It is difficult, however, to conclude which explanation is correct, since the proportion of gut contents was not determined through the season. The nutritional implications of gut contents were investigated, however, in late season caterpillars. The overall protein content of caterpillar gut contents (hereafter referred to as 'caterpillar guts') was considerably lower than that of the caterpillars' bodies minus gut contents (hereafter referred to as 'caterpillar bodies'), being 17.8% and 43.3% respectively. From Figure 2.9a, it can be seen that all amino acids, with the exception of hydroxyproline, were considerably less abundant in caterpillar guts compared with caterpillar bodies, in terms of mass per unit dry mass. If we consider protein quality, however, by examining amino acid composition in terms of mass per unit protein mass (Figure 2.9b), it is apparent that the levels of most amino acids were very similar in both caterpillar guts and caterpillar bodies: the main exceptions being hydroxyproline, alanine and cysteine, which were higher in caterpillar guts, and tyrosine and lysine which were lower in caterpillar guts. Of these, only lysine is an EAA with cysteine and tyrosine being SEAA.

Plant-derived protein generally has quite different proportions of EAA from animal proteins: in particular, lysine and the SAA (especially methionine) are usually present in significantly lower levels in plant-derived proteins. However, in our analyses of caterpillar guts and caterpillar bodies, proportions of amino acids were very similar; the proportion of lysine being only slightly higher in caterpillar bodies; levels of methionine being equal; and the proportion of cysteine actually being somewhat higher in caterpillar guts. This is quite surprising, since one would expect the gut contents to consist almost entirely of oak leaf tissue.

Although we have no amino acid analysis data for oak leaves, there is no obvious reason to suspect that they should depart radically from the pattern seen in other plant material. How, then, can one explain the observed result?

Caterpillars have a fairly rapid throughput of leaf material and probably, therefore, only extract a proportion of all the amino acids present. If amino acids were not digested/absorbed equally, and digested amino acids remained in the gut for a short time after the semi-digested material had passed on, then the overall composition of amino acids present in the gut at any one time would be different from that of leaf material. This would be exacerbated if, in the short period of time between caterpillars being dislodged from the foliage and being killed, they excreted a portion of the partially digested leaf material, without ingesting any fresh material. Also, the caterpillar will have contributed digestive enzymes to gut contents, adding unknown levels of protein. The protein content of early growth in grasses, forbs and browses can be as high as 20-30% of dry weight; falling to as little as 3-4% at maturity (Robbins 1993). Thus, although the protein content of 17.8% found for caterpillar guts is consonant with them consisting predominantly of oak leaf fragments, one cannot rule out the possibility that the caterpillar guts contained a significant and selective proportion of digested amino acids and digestive enzymes. A second possibility is that, as a result of freezing and thawing prior to dissection, many caterpillar body cells may have been ruptured allowing higher quality cytoplasmic contents to 'contaminate' gut contents. It should be noted that these hypotheses are not mutually exclusive.

To return again to the full range of invertebrates examined, it is apparent from figure 2.8b that relative amino acid compositions are very similar between both groups of spiders and all three stages of Lepidopteran larvae, with the striking exception of taurine (of the other amino acids, most variation is seen in serine, proline, tyrosine and arginine):taurine levels in wolf spiders were over 100 times the mean level in caterpillars; whilst in small arboreal spiders, taurine levels were close to 40 times the level in caterpillars. Taurine was only detected in relatively small quantities in the blue tit eggs. However, conjugation of bile acids with taurine, in the formation of bile, occurs obligatorily in most vertebrates above the selachians; with the exception of some mammals which can also use glycine (Jacobsen & Smith 1968; Haslewood 1962). In the majority of vertebrates studied, the taurine required can be synthesised in the body from cysteine; which can in turn be synthesised from methionine (Jacobsen & Smith 1968). Therefore, although taurine is not usually an essential nutrient in the diet, it can, if present, have a sparing effect on the animal's cysteine/methionine requirements (Scott, Nesheim & Young 1982); thus freeing-up more of the birds dietary SAA intake for egg formation. An additional, and potentially very important, role for dietary taurine in the development of post-hatching young is discussed in Appendix 1.

The comparison of amino acid requirements during laying, and the amino acid composition of small spiders and caterpillars (Table 2.4c,d) suggests that SAA would most likely be the most limiting EAA in the diet of a laying blue tit; in agreement with the predictions of Murphy (1994). Although methionine levels are similar between invertebrate protein and blue tit egg protein (Figures 2.8a,b), cysteine levels are significantly greater in egg protein compared with invertebrates (151% to 201% of invertebrate level for wolf spiders and late season caterpillars, respectively). Although spider protein was a somewhat better source of cysteine than caterpillar protein, SAA would still appear to be the most limiting EAA in a spider based diet. As discussed above, the high levels of taurine available in spiders could probably substitute for a proportion of the birds maintenance requirements for SAA, although there is no information available regarding exactly what proportion of total SAA requirements in a blue tit could be satisfied by dietary taurine. However, I believe it is unlikely to be particularly substantial and, consequently, SAA would still be the most limiting EAA.

In the above considerations, a utilisation efficiency of 75% was assumed for all dietary protein. However, there is some evidence that the digestibility of adult insects may be less than for invertebrate larvae as a consequence of the greater proportion of cuticle (Karasov 1990). Thus, although the total protein content of spiders was higher than for caterpillars, available protein levels may be more similar.

Amino acid composition was only determined for Lepidopteran larvae and Araneae. Although these were the most abundant invertebrates available at identified foraging sites, a small proportion of other arthropods, predominantly other bud/leaf inhabiting larvae, were also present and may have featured in the diet. Although I have no data on the amino acid composition of these other larvae, it is probable that a small insect larvae feeding exclusively on oak bud tissue will have a somewhat similar amino acid composition to a Lepidopteran larvae feeding on the same material. Consequently, the inclusion of a small proportion of other larvae in the diet is unlikely to affect our findings. Similarly, plant protein generally contains a comparatively low proportion of SAA and, as a result, the ingestion of any oak bud tissue would be unlikely to alter our conclusions regarding SAA being the most limiting EAA during egg laying.

# CHAPTER 3

## Nutritional constraints on egg production in the blue tit : a supplementary feeding study

*"The birdwatcher .....may have occasion to use more specialised instruments for specialised work. These may include traps, stuffed and dead birds, dummy eggs, nestboxes, paint, mirrors, golf-balls, little cakes, tin plates, string, climbing irons, boats, motor-bicycles, balloons, kites, aeroplanes, money, guns, and butter."*

James Fisher (1940, *Watching Birds*)

## 3.1 Introduction

The production of eggs is a nutritionally demanding process for many birds, particularly small passerines, some of whom lay a clutch of eggs weighing more than the females' own body weight. The influence of food supply prior to and during the egg laying period on egg production has been examined in a variety of bird species (review in Martin 1987). Purely observational, correlative studies have often found a positive relationship between favourable food conditions and reproductive parameters such as date of laying and clutch size (for example Perrins 1965; Murphy 1986; Jarvinen & Vaisanen 1984; Perrins & McCleery 1989; Perrins 1991). These studies suggest that food availability can limit egg production, and evidence for this comes from experimental studies involving the provision of supplementary food. These have generally found an advancement of laying date but much less frequently any increase in clutch size (Reviews in Martin 1987; Arcese & Smith 1988; Boutin 1990). Effects on egg size and weight are less frequently recorded, but where they have been measured, most found no increase.

Most of these experimental studies have assumed that it is the energy content of the diet that is limiting and few studies have considered the nutritional quality of supplementary feeding. However, Magrath (1992) and Bolton, Houston & Monaghan (1992) have suggested that birds may have requirements for specific nutrients during egg production, such as essential amino acids, and that limited availability of these in the diet may constrain egg size. A field study by Bolton *et al.* (1992) on the lesser black-backed gull (*Larus fuscus*), involving the provision of supplementary foods of differing nutritional composition, but equivalent calorific value, during the pre-laying period, demonstrated that egg production could be differentially affected by the protein quality of the supplementary food provided. Experimental work with captive zebra finches (*Taeniopygia guttata*) has further demonstrated the importance of diet quality on egg production and suggests that an imbalance in the amino acid composition of egg proteins compared to those present in the diet may result in access to quality protein having an important influence on egg production (Houston *et al* 1995a; Selman & Houston 1996).

The factors influencing egg production have been most extensively studied in tit species, in which over eight supplementary feeding trials have been conducted. Although the type of food used has varied between studies, very little attention has been paid to the comparative effect of more than one type of diet.

This chapter reports the results of an experimental field study to compare the effects of different food quality on egg production in a population of blue tits (*Parus caeruleus* ). Supplementary food was provided prior to, and during, egg production to examine whether

increased food supply could enhance egg production and, if so, whether any such enhancement was due simply to energy limitation or to a more specific nutrient requirement. To achieve this, one experimental group received a food supplement composed only of animal fat whilst the other experimental group received a food supplement containing cooked hens' eggs together with animal fat. A third control group received no food supplement. Since it is likely that all specific nutrients required for egg production would be present in the egg food supplement, any nutritional limitation which exists on egg production should be released in this group and egg production enhanced. If a similar enhancement of egg production occurred in the group receiving animal fat this would indicate that it was energy level of the diet which was limiting.

## 3.2 Methods

All field studies were carried out on a population of blue tits using nestboxes in an oak-dominated woodland around the University Field Station, Rowardennan, Loch Lomond, North of Glasgow.

### 3.2.1 Feeding schedule

All nestboxes were checked weekly for signs of nestbuilding, beginning in late March. When a new nest was found, which was at least one quarter built, it was assigned to either one of the experimental groups or to the control group. Supplementary food was provided from the following day in wiremesh feeders suspended close to the chosen nestbox. Feeders were replenished weekly, or when nearly empty if this occurred within one week, so that supplementary food was provided *ad libitum*. Feeding continued until clutch completion. Control birds received no additional food. The fat diet consisted of blocks of lard. The egg protein diet consisted of 100 hens' eggs minus their shells (approx. 5.5kg) : 500g lard : 500g oatmeal heated together: earlier trials had shown that tits would not feed on a diet of pure egg and the cooked egg/lard/oatmeal mix was found to be most palatable in a series of experimental choice trials using various dietary mixes. Approximately 200g of lard or 500g of egg/lard/oatmeal mix was supplied per feeder when deployed or refilled: the greater quantity in the egg mix feeder was to allow for greater wastage through spilling and dehydration.

Blue tits usually become territorial five to six weeks before laying and continue territoriality until chick rearing (C.M. Perrins, personal communication). Supplementary feeding at a nestbox began, on average, some two to three weeks before the onset of laying and observations suggested that tits from neighbouring territories were denied access to the feeders, which were used exclusively by the intended nesting pair.

### 3.2.2 Assessment of reproductive parameters

Once a nest was fully built, it was checked daily in order to determine the onset of laying. After the first egg was laid, a nest was visited every second day, until clutch completion. At each visit any new eggs were numbered, using permanent OHP pens, weighed (to 0.05g) with a 5g pesola spring balance and the length and width measured (to 0.05mm) with vernier callipers. Egg volume was calculated using the equation : Egg Volume = $0.51 \times$ egg length $\times$ egg width$^2$ (from Hoyt 1979).

If a female had not begun incubation by clutch completion, the nest continued to be checked daily to determine the onset of incubation. Nests were then not visited for 10 days, after which they were visited daily to determine hatching date. After the first egg had hatched, a nest continued to be checked daily until all the viable eggs had hatched. Hatching asynchrony was calculated as number of days between hatching of first chick and last chick. The duration of incubation was calculated as the time from determined onset of incubation to hatching of the first chick. The delay in onset of incubation in relation to clutch completion was calculated as the number of days after clutch completion before incubation began. Therefore, if incubation began before clutch completion, incubation delay will have a negative value. Nests were visited again when the chicks were 7 and 14 days old. If hatching was asynchronous, the nest was visited when ≥50% of the chicks were of the required age. When the chicks were 7 days old they were weighed (to 0.1g) using a 50g pesola spring balance, and measurements taken of wing length, radius & ulna length and tarsus length (to 0.05mm) using a standard wing rule. When the chicks were 14 days old they were weighed again and measurements taken of tarsus length and wing length. Young were then ringed using an individual metal BTO ring on one leg and a red plastic ring on the other. Blue tits fledge at approx. 18 days, therefore nests were visited at approx. 25 days after hatching to check if all young had fledged.

## 3.3 Results

### 3.3.1 Onset of laying

Onset of laying data was square-root transformed, in order to improve normality, prior to performing parametric analysis. The mean laying dates in days from April 29$^{TH}$ are presented in Figure 3.1, which shows that the egg-fed and lipid-fed treatment groups laid significantly earlier than the control group (Anova, F = 3.376, d.f. = 2,62, $p<0.05$; Duncans Multiple Range Statistic, egg-fed group and fat-fed group differ significantly from control).

### 3.3.2 Egg production

The eggs within a clutch are not statistically independent (variation in egg size and weight was considerably greater between clutches than within, F-test, $p<0.00005$ in all cases) and, therefore, analyses of egg size between feeding treatments were carried out on clutch means.

Figure 3.2a shows mean values for egg volume. There was a significant difference in egg volume between treatments (F = 3.570, d.f. = 2,62, $p<0.05$), however, only in the birds fed with the egg supplement were the eggs significantly larger than control (Scheffe test).

A similar effect to egg volume was also seen for egg weight, although the difference was not statistically significant ($p = 0.08$): this may simply be due to less precise measuring of egg weight (which was measured to 0.05g, approx. 5% of mean value compared to egg length/width measurements which were to 0.05mm, approx.0.5% of mean value).

Figure 3.2b shows the effect of laying sequence on mean egg volume in the three treatment groups (eggs laid after the 11$^{th}$ egg in a sequence have been omitted, as these are only represented by a few nests). Egg volume appears to fall noticeably through the laying sequence (at least in the first 7 eggs) in the egg-fed group, whilst it declines only slightly or not at all in the fat-fed and control groups. However, mean egg volume and egg mass in the egg-fed group remains greater than in controls, throughout the laying sequence.

As mentioned earlier, the individual eggs cannot be regarded as independent data points, in an analysis of several clutches. Therefore, simple linear regression equations were calculated, which describe the relationship between egg volume and laying sequence for each clutch. The slope value obtained for each clutch was then used in the subsequent analyses. The mean slope was negative in all three treatment groups but only in the egg-fed treatment group did it differ significantly from zero (Table 3.1). Despite this, mean slope did not differ significantly between treatment groups in an Anova.

**Table 3.1**: Mean (± S.E. (n)) for slope of intraclutch regressions of egg volume through the laying sequence.

| | Egg Supplement | Lipid Supplement | Control | Statistics |
|---|---|---|---|---|
| Slope | -6.16± 2.74 | -1.74± 2.34 | -1.42± 2.38 | $F_{2,62} = 0.97$ |
| | (17) | (19) | (29) | n.s. |
| Significance of slope | $t_{16} = -2.24$ | $t_{18} = -0.74$ | $t_{28} = -0.59$ | |
| | $p<0.05$ | n.s. | n.s. | |

There are two reasons for also considering the intercepts of these intraclutch laying sequence regressions. Firstly, if egg size declines through the laying sequence, then a female laying a large clutch ($n + x$) would have a smaller *mean* egg size than a female laying a smaller clutch ($n$), as a result of the additional 'x' smaller eggs at the end of the laying sequence, even if the sizes of the first '$n$' eggs in the two clutches were identical. Using intercepts overcomes this problem. Secondly, if the slope of the regression of egg volume through the laying sequence is negative and differs between treatments, then the magnitude of the difference in absolute egg volume between treatment groups will be greater (and therefore more likely to be detected statistically) early in the laying sequence. Using intercepts allows us to examine the situation at the beginning of the laying sequence.

Figure 3.2e shows mean values for the intercepts of intraclutch regressions of egg volume through the laying sequence. There was a significant difference in mean intercept between treatments ($F = 4.316$, d.f. = 2,62, $p<0.02$) with the intercept in the egg-fed group being significantly greater than the intercept in both the fat-fed and control groups (Duncans multiple range statistic). We can therefore conclude that egg-fed birds laid eggs which were significantly larger (about 7%) than those of the controls, and the first laid eggs in their clutches were significantly larger than those of both the fat-fed group and the controls.

Clutch size data were transformed by squaring, in order to normalise it, prior to performing parametric analysis (Mean clutch size +S.E. (n) : egg-diet 9.73 +0.61 –0.70 (17), fat-diet 9.78 +0.60 –0.62 (21), control 9.55 +0.33 –0.36 (29); $F = 0.103$, d.f. = 2,64, n.s.). Clutch size, however, declined significantly through the season (Pearsons $p<0.005$, n=65; $p<0.05$, n=27 for controls only). In order to control for this, an analysis of covariance was carried out with laying date as a covariate. Clutch size was unaffected by supplementary feeding. ($F = 0.175$, d.f. = 2,64, n.s.)

### 3.3.3 Incubation

There were no significant differences between experimental groups in the onset of incubation (Mean date in days from 29$^{TH}$ April ± S.E. (n) : egg-fed 16.2 ±0.9 (13), lipid-fed 17.3 ±1.0 (16), control 18.1±0.8 (23), F = 2.715, d.f. = 2,64, n.s.), duration of incubation period (Mean in days ± S.E. (n) : egg-fed 12.8 ±0.5 (12), lipid-fed 13.5 ±0.5 (15), control 13.0 ±0.3 (20), F = 0.452, d.f. = 2,45, n.s.) or delay in the onset of incubation in relation to clutch completion (Median delay in days [interquartile range] (n) : egg-fed 0.0 [−1.0 - 2.5] (13), lipid-fed 0.0 [−1.0 - 2.75] (16), control 0.0 [ 1.0 - 1.0] (20), $\chi^2$ = 0.222, d.f. = 2, n.s.).

### 3.3.4 Hatching and chick parameters

There were no significant differences in hatching date (Mean date in days from 29$^{TH}$ April ± S.E. (n) : egg-fed 30.6 ±1.2 (14), lipid-fed 30.2 ±0.7 (16), control 33.0 ±1.1 (23), F = 2.218, d.f. = 2,50, n.s.), hatching success (Median [interquartile range] (n) : egg-fed 0.85 [0.00 - 1.00] (17), lipid-fed 0.86 [0.33 - 0.90] (21), control 0.89 [0.00 - 1.00] (29), $\chi^2$ = 0.211, d.f. = 2, n.s.) or hatching asynchrony (Mean ± S.E. (n) : egg-fed 1.2 ±0.2 (14), lipid-fed 1.2 ±0.2 (16), control 1.5 ±0.2 (23), F = 0.971, d.f. = 2,50, n.s.) between the 3 experimental groups.

There were no significant differences between treatments in brood size at 7 days (Mean ± S.E. (n) : egg-fed 8.5 ±0.9 (12), lipid-fed 8.2 ±0.6 (16), control 8.3 ±0.6 (19), F = 0.055, d.f. = 2,44, n.s.) and 14 days ( egg-fed 8.5 ±0.9 (12), lipid-fed 7.9 ±0.5 (16), control 8.3 ±0.6 (19), F = 0.180, d.f. = 2,42, n.s.) or fledging number (as at 14 days). As with clutch size, fledging number declined through the season; fledging number was significantly negatively correlated with hatching date (Pearsons, $p<0.0005$, $n=45$; $p<0.01$, $n=18$ for controls only). In order to control for this, an analysis of covariance was carried out with hatching date as a covariate. Fledging number was unaffected by supplementary feeding (F = 0.757, d.f. = 2,44, n.s.).

Chick size measurements (weight, tarsus, radius & ulna and wing) at 7 and 14 days are given in Table 3.2. There were no significant differences in these measurements between the experimental groups.

**Table 3.2**: Mean (± S.E. (n)) for chick measurements at 7 and 14 days old.

| | Egg Supplement | Lipid Supplement | Control | Statistics |
|---|---|---|---|---|
| 7 Day Chick Weight (g) | 7.84± 0.22 | 7.76± 0.22 | 7.52± 0.32 | $F_{2,44} = 0.692$ |
| | (12) | (16) | (19) | n.s. |
| 7 Day Chick Tarsus (mm) | 16.63± 0.29 | 16.18± 0.24 | 15.79± 0.37 | $F_{2,44} = 1.608$ |
| | (12) | (16) | (19) | n.s. |
| 7 Day Chick Radius&Ulna (mm) | 14.97± 0.23 | 14.72± 0.20 | 14.35± 0.36 | $F_{2,44} = 1.059$ |
| | (12) | (16) | (19) | n.s. |
| 7 Day Chick Wing (mm) | 15.77± 0.57 | 15.21± 0.34 | 14.80± 0.60 | $F_{2,44} = 0.794$ |
| | (12) | (16) | (19) | n.s. |
| 14 Day Chick Weight (g) | 11.57± 0.29 | 11.59± 0.23 | 11.75± 0.23 | $F_{2,42} = 0.165$ |
| | (11) | (16) | (18) | n.s. |
| 14 Day Chick Tarsus (mm) | 20.12± 0.12 | 20.01± 0.11 | 20.12± 0.13 | $F_{2,42} = 0.275$ |
| | (11) | (16) | (18) | n.s. |
| 14 Day Chick Wing (mm) | 45.54± 0.73 | 42.20± 0.64 | 40.98± 1.07 | $F_{2,42} = 0.862$ |
| | (11) | (16) | (18) | n.s. |

There was also no difference in survivorship (the proportion of hatched eggs which fledged) between the experimental groups (Medians [interquartile range] (n) : egg-fed 1.00 [0.91 - 1.00] (12), lipid-fed [1.00 [0.89 - 1.00] (16), control 1.00 [0.89 - 1.00] (18), $\chi^2 = 0.608$, d.f. = 2, n.s.).

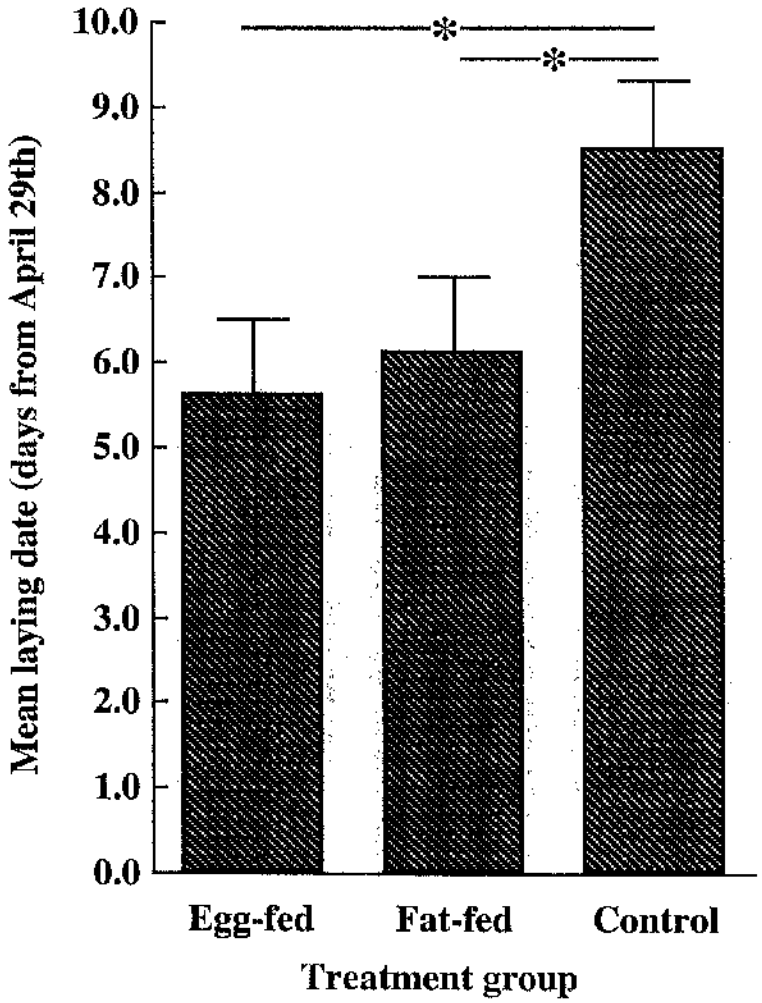



**Figure 3.1**: Mean values (+ SE) of onset of laying for control ($n$=27), lipid-fed ($n$=21) and egg-fed ($n$=17) blue tits. Significance of pairwise comparisons indicated, *$P$<0.05.

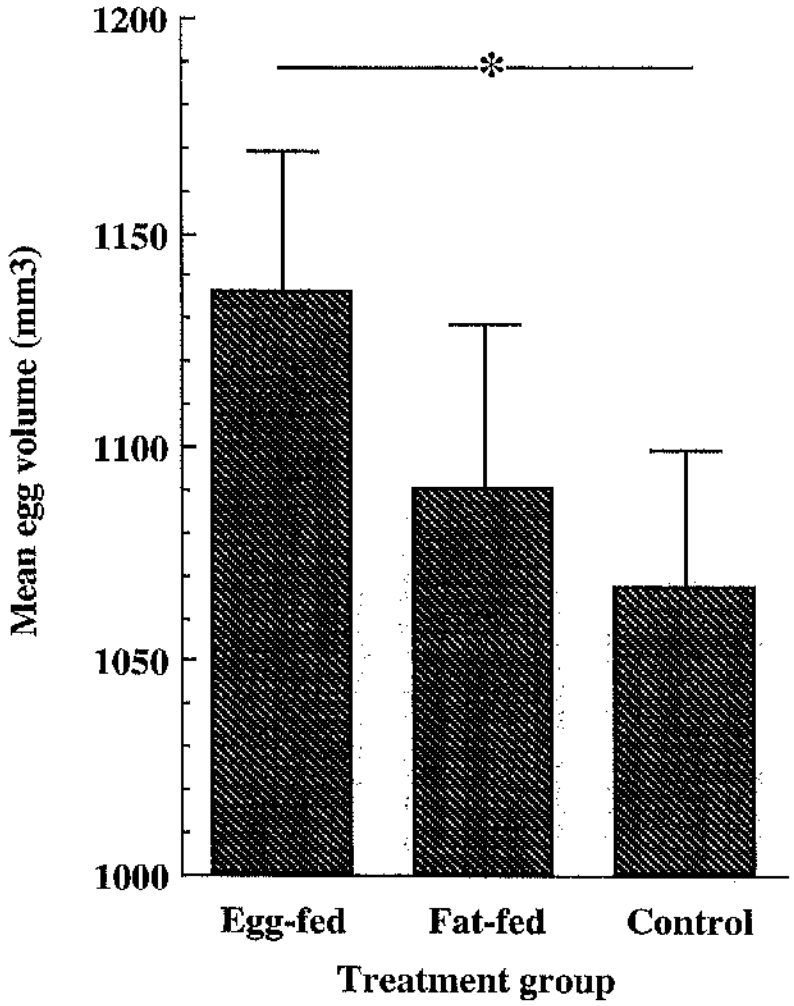



**Figure 3.2a**: Mean values (+ SE) of egg volume for eggs laid by control ($n$=29), lipid-fed ($n$=19) and egg-fed ($n$=17) blue tits. Significance of pairwise comparisons indicated, *$P$<0.05.

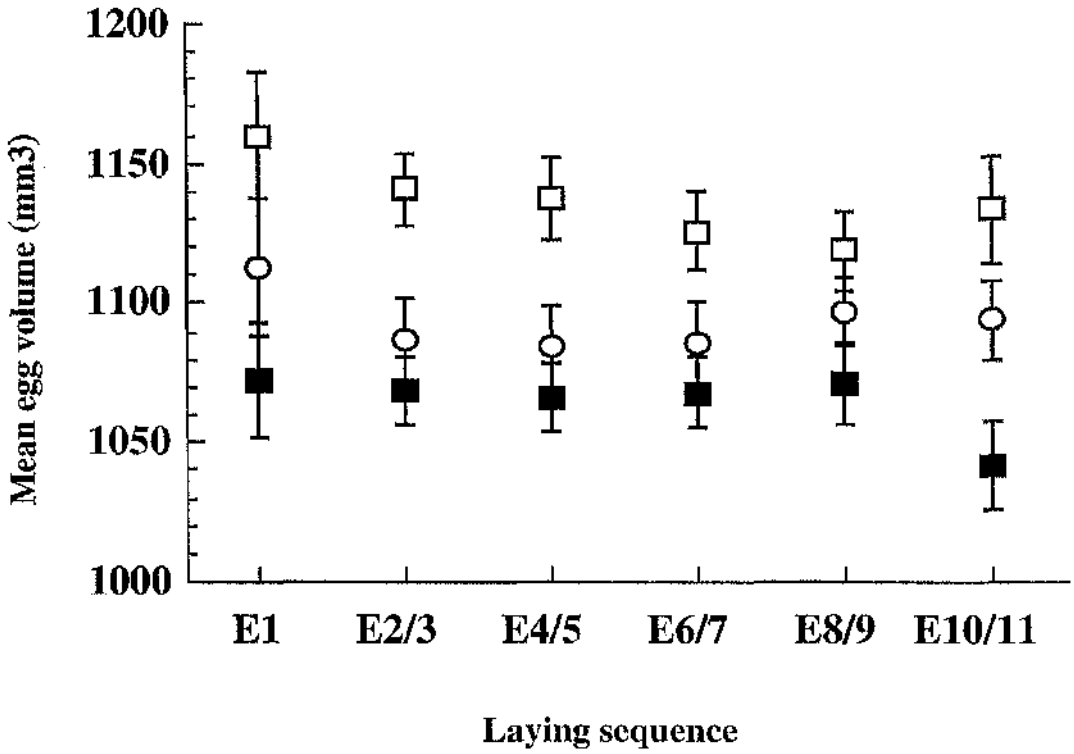



**Figure 3.2b**: Mean values (+ SE) of egg volume through the laying sequence, for eggs laid by control ( ■ ,*n*=29 clutches), lipid-fed (○ ,*n*=19 clutches) and egg-fed ( □ ,*n*=17 clutches) blue tits. E1 indicates the first egg laid in the clutch. E2/3 indicates the mean for second and third laid eggs.

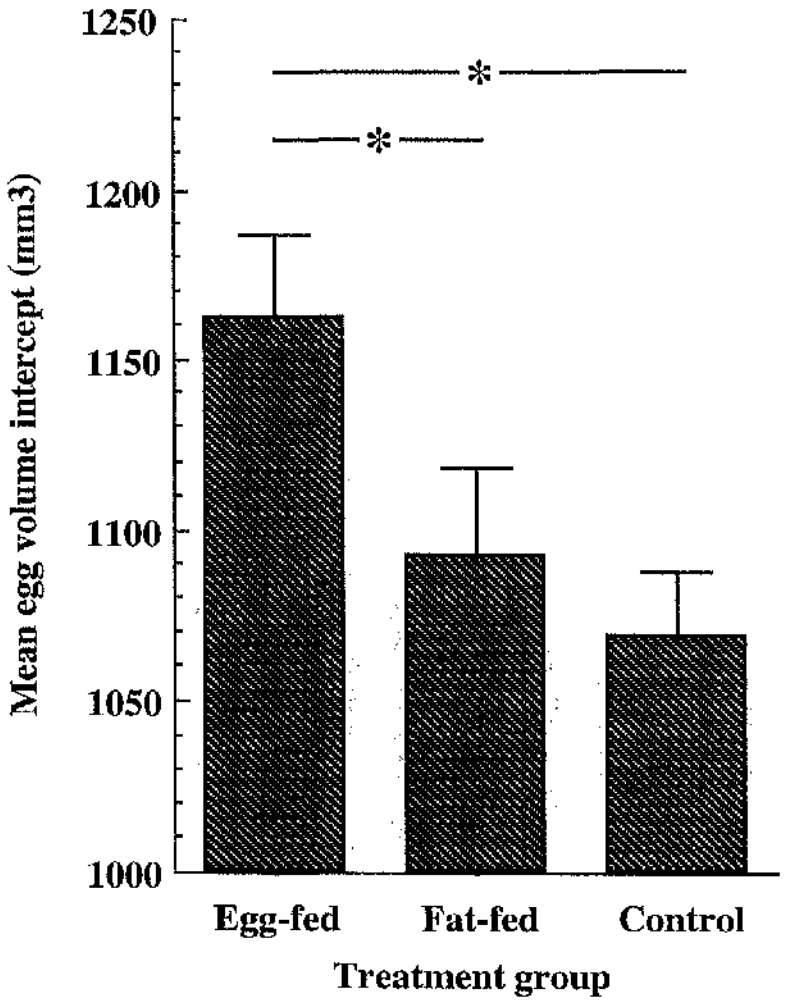



**Figure 3.2c**: Mean values (+ SE) of intercepts, for intraclutch regressions of egg volume through the laying sequence, for control ($n$=29), lipid-fed ($n$=19) and egg-fed ($n$=17) blue tits. Significance of pairwise comparisons indicated, *$P$<0.05.

## 3.4 Discussion

FOOD SUPPLY AND TIMING OF BREEDING.

The onset of laying was significantly advanced by the provision of supplementary food. The mean advancement of approximately $2^1/_2$ days is comparable with that produced in other supplementary feeding studies involving blue tits; 5 and 6 days in different years in Clamens & Isenmann's (1989) study and 4 days and 6 days in Nilsson & Svensson's (1993a) and Nilsson's (1994) study. It is known that there is considerable variation, between years and study areas, in the degree of laying advancement induced by supplementary feeding. The extent of the effect correlates inversely with the natural food supply, so that experimental feeding generally has greatest effect in years when natural feeding is poor (Martin 1987) or in low quality territories (Svensson & Nilsson 1995). With an advancement of only $2^1/_2$ days it seems likely that natural food supplies at our study site were relatively good during the period of the study.

Food supply is almost certainly only one of several factors which act on a female blue tit to determine the onset of laying. Photoperiod (Suomailainen 1937; Farner & Wingfield 1980), environmental temperature (Kluyver 1952; Lack 1958), female age/experience (Dhondt 1989; Perrins & McCleery 1985; Nakamura 1995), breeding density (Arcese & Smith 1988), genetics (van Noordwijk 1984; Blondel *et al* 1990) and body condition (Murphy 1986) have all been shown to influence timing of breeding in passerines. Despite this, advancement of laying date is the most common response to supplementary feeding (Martin 1987; Arcese & Smith 1988; Boutin 1990). Savings of time, leading to earlier hatching, appear to be extremely important in increasing fitness. Norris (1993) demonstrated a causal link between hatching date and chick survival in blue tits, independent of any parental quality effects; chick survival decreased with laying date, although survival was also reduced if chicks hatched very early. There are two probable main advantages of early laying and hatching:

1) That nestlings/fledglings are present at a time when more food is available. In many species, particularly Parids, the prey abundance peaks relatively early in the year in relation to the birds' breeding cycle, with the result that late nestlings/fledglings are present after the food peak (Perrins 1970). Early hatching has been shown to positively influence the survival of young (vonHaartmann 1966; Perrins 1979; Newton & Marquiss 1984).

2) Juveniles which are present earlier in the season may be socially dominant over those hatching later (Garnet 1981; Arcese & Smith 1985) and have increased establishment success (Nilsson & Smith 1988b; Nilsson 1989, 1990), which leads to an increased probability of being recruited into the breeding population (Hochachka 1990).

There was no significant difference in the degree of advancement between the experimental group receiving supplementary food composed of fat and the group receiving a protein diet. This would suggest that the food constraint on onset of laying is purely an energetical one. Perhaps a female must delay the onset of laying until she can obtain sufficient energy to allow egg synthesis whilst maintaining her own metabolic requirements (**Constraint hypothesis**: Perrins 1970). Alternatively, food levels may not directly constrain onset of laying but, rather, may act as a cue to the female, indicating size and timing of food levels at some important stage of the breeding cycle in the future eg. brood rearing (**Cue hypothesis**: Lack 1954; Kallander & Karlsson 1993). This point of uncertainty has been noted by several researchers studying the interaction between food supply and breeding.

The majority of supplementary feeding studies only manage to advance laying by a relatively small amount in comparison to the magnitude of differences in mean onset of laying seen between years. This suggests that, regardless of whether energy supply acts as a cue or a proximate constraint, once food levels pass a threshold level, onset of laying is determined by other environmental factors.

The time advantage of feeding, as evidenced by earlier laying dates, had vanished, however, by the time of onset of incubation. This is a similar result to that obtained by Nilsson (1994). Female blue tits in our supplementary fed treatment groups may have postponed the onset of incubation because they were proximately constrained by food abundance/energy intake rate and had to wait until they passed some intake threshold allowing them to incubate successfully, or alternatively, they may have been waiting for food abundance/energy intake rate (in conjunction with other environmental cues) to pass a threshold indicating that beginning incubation now would result in the chicks hatching at an appropriate time for rearing.

## FOOD SUPPLY AND CLUTCH SIZE

The provision of supplementary food had no effect on clutch size in our study. A few other studies have found increased clutch size with supplementary feeding ,but these are in the minority (eg. Högstedt 1981; Nilsson 1991). In contrast, many non-manipulative studies have demonstrated that clutch sizes are larger in more favourable food years or habitats (from Martin 1987). As Martin (1987) points out, most studies relating natural food availability and clutch size, either use very different years or are conducted over a relatively long time scale and thus have a higher chance of including years of low food availability when clutch size may be depressed. On the other hand, experimental studies are typically conducted over just one or a few years, and are therefore more likely to contain average years when natural food levels may be above the threshold at which clutch size ceases to be depressed by food availability and

is, instead, controlled by other proximate factors such as date (Daan et al 1988) or predation risk (Lima 1987). Note that the threshold level of food need not be the same for onset of laying and clutch size determination. As with onset of laying, low food levels may result in reduced clutches directly by providing insufficient nutrients to form a larger clutch, or may act indirectly as a cue that food levels at some future stage will be insufficient to raise a larger brood.

VARIATION IN EGG SIZE IN RELATION TO SUPPLEMENTARY FEEDING.

Those females receiving supplementary food composed of fat produced slightly, but not significantly, larger eggs (2% increased mean volume) than control females. However, the provision of supplementary food containing egg protein resulted in a significant increase of 7% in the mean volume of eggs. The eggs laid early in the laying sequence show the greatest size increase, with eggs from the egg-fed treatment group being significantly larger than those in both the fat-fed and control groups.

It would appear, therefore, that there is a nutrient (or nutrients) present in eggs which is normally limited in the blue tits' diet, and which, if provided, can result in the production of larger eggs. The difference between the experimental groups suggests that this nutrient may not be just lipid ie. the limitation is not simply energetical. This is particularly interesting when considered with the fact that both the supplementary food composed of fat and the supplement composed of eggs and fat produced the same degree of laying enhancement. This would suggest either of two possible scenarios. Energy levels could be acting as a cue for the onset of laying and egg production/egg size is proximately constrained by the availability of a specific nutrient(s) (the slight increase in egg size in the fat-fed group could either be a chance effect or the extra energy could have allowed selective foraging for the limiting nutrient(s)). Alternatively, onset of laying and egg production could be, initially, proximately constrained by energy availability but if the energy constraint is removed, egg size can only be increased by a very small amount (without detrimentally altering egg composition) before other nutrient(s) become limiting.

What then, could this limiting nutrient be? The other major component of eggs, aside from lipid and water, is protein and, therefore, this is a possible limiting factor. However, blue tits are predominantly insectivorous and, therefore, the proportion of protein in their diet, in relation to lipid, should be high. If this is the case, then it seems unlikely that protein, generally, could be limiting whilst lipid is not. It is possible, however, that only a few essential amino acids are actually limiting. It is known that the amino acid composition of egg proteins differs from that of animal tissues, and there are a few amino acids (the sulphur amino acids

and lysine in particular) that are represented in higher proportions in eggs (Murphy 1994; Houston, Donnan & Jones 1995a). Egg production could potentially be restricted by the availability of a few or even one essential amino acid. However, further research would be needed to investigate this and from the current study we can only suggest that there is some unknown nutrient which appears to limit egg production.

## THE SIGNIFICANCE OF EGG SIZE

In order for egg size to have any effect on parental fitness, an increase in egg size must result in enhanced hatchability, higher survival of young or greater probabilities for the young to become established (Nilsson & Svensson 1993b). A few studies have reported reduced hatchability of, particularly, small eggs (eg. Rofstad & Sandvik 1985; Perrins 1996). In a study by Nilsson & Svensson (1993b) on the blue tit, a significant positive relationship was found between egg mass and hatching mass and Perrins (1996) reported that in an extensive data set on Great tits (*Parus major*, L), fledging weight increased with egg weight and recruitment to the breeding population was closely related to fledging weight (Perrins 1965).

Many studies, however, can provide, at best, only equivocal support for a positive relationship between egg size and offspring fitness. This can be, as Mueller (1990) pointed out, because of the confounding effect of intraclutch egg size variation in the laying sequence and concurrent differential mortality due to asynchronous hatching and brood reduction, rather than egg size *per se*. A more common, and often noted, problem is the failure to control for confounding factors such as parental quality, which may be correlated both with egg production and offspring fitness. A number of studies have controlled for one, or a number, of these potentially confounding variables and a few have still demonstrated a residual effect of egg size on chick fitness (Nisbet 1978; Magrath 1992; Bolton 1991).

A smaller number of studies have examined the relationship between egg size and chick size, growth and/or survival in the early stages of chick rearing, prior to fledging. The majority of these have found a positive relationship between egg size and early chick size, growth and survival (eg. Schifferli 1973; Jarvinen & Ylimaunu 1984). A few, however, found that this relationship could not be detected when overall survival to fledging was considered.

Thus, egg size may have more importance to chick growth or survival early in chick rearing. As Williams (1994) points out, "slower growth immediately after hatch, due to hatching from a smaller egg, may be compensated for later in the rearing period" (Ojanen 1983a; Schifferli 1973; O'Connor 1975a). "However, early chick mortality cannot be compensated for (even though the effect can be swamped by subsequent egg size independent mortality, making its detection difficult or impossible)."

If egg size influences early chick growth or resistance to starvation or chilling, then egg size effects may be more apparent in years of low food availability or adverse weather conditions (Williams 1994). Ojanen (1983) and Davis (1975) both found a relationship between egg size and chick survival in some years and not others, although no data are given on prevailing environmental conditions. As our study was probably carried out in a relatively good food year, this may explain why no difference in offspring fitness was found between the treatment groups.

We can conclude that food quality may be an important factor in the proximate control of reproductive output. Most previous studies have only considered food supply as an energetic constraint in life history decisions. This study suggests that the quality of diet may also be significant and that it may act in a different manner on egg production to overall energy availability.

# CHAPTER 4

## Protein quality as a proximate constraint on egg production in the blue tit

*"There is eloquence in screaming"*

Patrick Jones

## 4.1 Introduction

Food supply is generally acknowledged to be one of the most important environmental factors determining the timing and level of investment in breeding by passerine birds. Purely observational, correlative studies have often found a positive relationship between favourable food conditions and reproductive parameters such as date of laying and clutch size (for example Perrins 1965; Murphy 1986; Jarvinen & Vaisanen 1984; Perrins & McCleery 1989; Perrins 1991). Further evidence for a causal relationship between food supply and reproductive output comes from studies involving the experimental provisioning of supplementary food. These studies have generally found an advancement of laying date but only sporadically any effect on egg size or number (Reviews in Martin 1987; Boutin 1990; Aparicio 1994). However, as pointed out by a number of researchers (Nisbet 1978; Krapu & Swanson 1975; Ewald & Rohwer 1982) differences in the quality of the supplementary food used may explain some of these inconsistencies. Magrath (1992) and Bolton, Houston & Monaghan (1992) have suggested that birds may have requirements for specific nutrients during egg production, and that limited availability of these in the diet may constrain egg production. If this is the case, then results of supplementary feeding experiments may depend to a large extent on the ability of the supplementary diet to provide the limiting nutrient(s).

A field study by Bolton *et al.* (1992) on the lesser black-backed gull (*Larus fuscus*), involving the provision of supplementary foods of differing nutritional composition but equivalent calorific value during the pre-laying period, demonstrated that egg production could be differentially affected by the nutritional quality of the supplementary food provided. Bolton *et al* (1992) proposed that this result was due to differing protein quality between diets, and that egg production may be limited by the availability of only a few specific amino acids. Experimental work with captive zebra finches (*Taeniopygia guttata*) has further demonstrated the importance of dietary protein quality on egg production and suggests that an imbalance in the amino acid composition of egg proteins compared to those present in the diet may result in access to quality protein having an important influence on egg production (Houston *et al* 1995a; Selman & Houston 1996).

We have previously demonstrated experimentally that whilst onset of laying in blue tits is influenced by dietary energy supply, egg size can be constrained by the availability of specific nutrients (Ramsay & Houston 1997; *Chapter 3*). Although the identity of these nutrients could not be ascertained it was proposed that the constraint may represent a requirement for high quality protein.

This chapter reports the findings of a second feeding experiment, to examine the extent to which egg production in blue tits is, in fact, constrained by the availability of high quality protein in the diet. As in the previous study, supplementary food was provided prior to and during the egg production period and subsequent reproductive output monitored. The supplementary diets used, were of identical calorific, 'crude' protein, and non-protein-nutrient content, but differing amino acid composition.

## 4.2 Methods

### 4.2.1 Supplementary diets

Ground peanuts were used as the base for both supplementary diets.

For the 'High quality' protein diet, Methionine, Lysine, Cysteine, Threonine and Tryptophan were added to the peanut base in order to produce a mixture with similar proportions (mg of amino acid per g Nitrogen) of these amino acids as would be found in hens' eggs (Paul & Southgate 1978; Paul *et al* 1978).

In the 'Low quality' protein diet, Glutamic acid was added to the peanut base in order to produce a mixture with the same total protein content as the 'High quality' protein mixture. The amounts of amino acids added are shown in Table 4.1.

**Table 4.1**: Masses of crystalline amino acids added to supplementary diets.

| | | Methionine | Lysine | Cysteine | Threonine | Tryptophan | Glutamic acid |
|---|---|---|---|---|---|---|---|
| 'High Quality' Protein Diet | Amino acids added (g/kg peanuts) | 8.8g | 13.9g | 2.3g | 9.9g | 2.7g | 0g |
| 'Low Quality' Protein Diet | Amino acids added (g/kg peanuts) | 0g | 0g | 0g | 0g | 0g | 37.6g |

L-lysine hydrochloride has a natural L-lysine potency of 78.4%. Therefore, 13.9g of L-lysine hydrochloride was added in order to add 10.9g of available lysine to the mixture. When expressing the amino acid content of the diet in terms of mg AA per 100g of material (Table 4.2), we consider that 13.9g of material has been added to the mixture, but that the lysine content has been raised by only 10.9g.

Similarly, DL-Methionine has a natural L-Methionine potency of 78 - 92% (we used a value of 85%). We therefore, added 8.8g of DL-Methionine in order to add 7.5g of available methionine to the mixture. As before, when expressing the amino acid content of the diet in

terms of mg AA per 100g of material, we consider that 8.8g of material has been added to the mixture, but that the methionine content has been raised by only 7.5g.

The amino acid compositions of the supplementary diets are shown in Table 4.2 and Table 4.3. Protein contents, calorific contents and energy : protein ratios are shown in Table 4.4. Energy : specific amino acid ratios are shown in Table 4.5.

### 4.2.2 Feeding Schedule

All nestboxes were checked weekly for signs of nestbuilding, beginning in late March. When a new nest was found, which was at least one quarter built, it was assigned to either one of the experimental groups or to the control group. Supplementary food was provided from the following day in wiremesh feeders suspended close to the chosen nestbox. Feeders were replenished weekly, or when nearly empty if this occurred within one week, so that supplementary food was provided *ad libitum*. Feeding continued until clutch completion. Control birds received no additional food.

Blue tits usually become territorial five to six weeks before laying and continue territoriality until chick rearing (C.M. Perrins, personal communication). Supplementary feeding at a nestbox began, on average, some two to three weeks before the onset of laying and observations suggested that tits from neighbouring territories were denied access to the feeders, which were used exclusively by the intended nesting pair.

### 4.2.3 Assessment of reproductive parameters

Once a nest was fully built, it was checked daily in order to determine the onset of laying. After the first egg was laid, a nest was visited every second day, until clutch completion. At each visit any new eggs were numbered, using permanent OHP pens, weighed (to 0.01g) with an Acculab Pocket Pro portable electronic balance and the length and width measured (to 0.05mm) with vernier callipers. Egg volume was calculated using the equation :

Egg Volume = 0.51× egg length × egg width$^2$ (from Hoyt 1979).

If a female had not begun incubation by clutch completion, the nest continued to be checked daily to determine the onset of incubation. Nests were then not visited for 10 days, after which they were visited daily to determine hatching date. After the first egg had hatched, a nest continued to be checked daily until all the viable eggs had hatched. At each visit any new hatchlings were weighed (to 0.01g) with an Acculab Pocket Pro portable electronic balance and individually marked using permanent OHP pens. The tufts of down above each eye and on top of the head could be dyed; these three sites and a range of colours allowing each chick to be

individually marked. Hatching asynchrony was calculated as number of days between hatching of first chick and last chick. The duration of incubation was calculated as the time from determined onset of incubation to hatching of the first chick. The delay in onset of incubation in relation to clutch completion was calculated as the number of days after clutch completion before incubation began. Therefore, if incubation began before clutch completion, incubation delay will have a negative value. Nests were visited again when the chicks were 7 and 14 days old. If hatching was asynchronous, the nest was visited when ≥50% of the chicks were of the required age. When the chicks were 7 days old they were weighed (to 0.01g) with an Acculab Pocket Pro portable electronic balance, re-marked by clipping a combination of claw tips, and measurements taken of wing length and tarsus length (to 0.05mm) using a standard wing rule. When the chicks were 14 days old they were weighed again and measurements taken of tarsus length and wing length. Young were then ringed using an individual metal BTO ring on one leg and a yellow plastic ring on the other. Blue tits fledge at approx. 18 days, therefore nests were visited at approx. 25 days after hatching to check if all young had fledged.

**Table 4.2**: Amino acid composition of foods (mg per 100g food)

| | Ile | Leu | Lys | Met | Cys | Phe | Tyr | Thr | Trp | Val | Arg | His | Ala | Asp | Glu | Gly | Pro | Ser |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whole raw hens' eggs | 690 | 1020 | 770 | 390 | 220 | 630 | 490 | 630 | 220 | 930 | 750 | 300 | 670 | 1320 | 1480 | 370 | 470 | 970 |
| Egg-based diet | 623 | 936 | 689 | 348 | 214 | 584 | 449 | 568 | 199 | 839 | 698 | 275 | 612 | 1195 | 1466 | 361 | 450 | 868 |
| 'High quality' diet | 854 | 1618 | 1869 | 960 | 530 | 1258 | 971 | 1537 | 530 | 1052 | 2831 | 611 | 971 | 2876 | 4611 | 1420 | 1097 | 1213 |
| 'Low quality' diet | 854 | 1618 | 890 | 288 | 324 | 1258 | 971 | 647 | 288 | 1052 | 2831 | 611 | 971 | 2876 | 7990 | 1420 | 1097 | 1213 |

All values obtained directly, or calculated from, Paul & Southgate, 1978; Paul *et al*, 1978.

**Table 4.3**: Amino acid composition of foods (mg per g Nitrogen)

| | Ile | Leu | Lys | Met | Cys | Phe | Tyr | Thr | Trp | Val | Arg | His | Ala | Asp | Glu | Gly | Pro | Ser |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whole raw hens' eggs | 350 | 520 | 390 | 200 | 110 | 320 | 250 | 320 | 110 | 470 | 380 | 150 | 340 | 670 | 750 | 190 | 240 | 490 |
| Egg-based mix | 344 | 517 | 381 | 192 | 118 | 323 | 248 | 314 | 110 | 464 | 386 | 152 | 338 | 660 | 810 | 199 | 249 | 480 |
| 'High quality' diet | 178 | 338 | 390 | 200 | 111 | 263 | 203 | 321 | 111 | 219 | 591 | 128 | 203 | 600 | 962 | 296 | 229 | 253 |
| 'Low quality' diet | 178 | 338 | 186 | 60 | 68 | 263 | 203 | 135 | 60 | 219 | 591 | 128 | 203 | 600 | 1667 | 296 | 229 | 253 |

All values obtained directly, or calculated from, Paul & Southgate, 1978; Paul *et al*, 1978.

**Table 4.4**: Macronutritional composition of supplementary diets and hens' eggs.

| | Protein content | Calorific content | | Energy : protein ratio | |
|---|---|---|---|---|---|
| | (g/100g ) | kcal/100g | kJ/100g | kcal/g protein | kJ/g protein |
| Whole hens' eggs | 12.3 | 147 | 612 | 12 | 50 |
| Amino acid supplemented diets | 26.4 | 581 | 2408 | 22.0 | 91 |
| Egg-based diet | 11.27 | 221 | 922 | 19.6 | 82 |

All values obtained directly, or calculated from, Paul & Southgate, 1978; Paul *et al*, 1978; Holland *et al* 1991.

**Table 4.5**: Energy : specific amino acid ratio for 'egg-based diet' and 'high quality protein' diet (kJ / 100g diet / g specific amino acid)

| | Ile | Leu | Lys | Met | Cys | Phe | Tyr | Thr | Trp | Val | Arg | His | Ala | Asp | Glu | Gly | Pro | Ser |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Egg-based diet **(A)** | 1.48 | 0.98 | 1.34 | 2.65 | 4.31 | 1.58 | 2.05 | 1.62 | 4.63 | 1.10 | 1.32 | 3.35 | 1.51 | 0.77 | 0.63 | 2.56 | 2.05 | 1.06 |
| 'High quality' diet **(B)** | 2.82 | 1.49 | 1.29 | 2.51 | 4.54 | 1.91 | 2.48 | 1.57 | 4.54 | 2.29 | 0.85 | 3.94 | 2.48 | 0.84 | 0.52 | 1.70 | 2.20 | 1.98 |
| **A / B** | 0.52 | 0.66 | 1.04 | 1.06 | 0.95 | 0.83 | 0.83 | 1.04 | 1.02 | 0.48 | 1.55 | 0.85 | 0.61 | 0.92 | 1.20 | 1.51 | 0.93 | 0.53 |

All values obtained directly, or calculated from, Paul & Southgate, 1978; Paul *et al*, 1978; Holland *et al* 1991.

## 4.3 Results

### 4.3.1 Onset of laying

The onset of laying in the two fed groups was slightly earlier than in the control group (Mean date in days from April 29$^{TH}$ ±SE ($n$):High quality protein, 6.0 ±1.2 (20); Low quality protein, 4.8 ±1.0 (18); Control, 8.4 +1.2 (20)) but, this difference was not statistically significant in an Anova ($F = 2.47$, d.f. = 2,55, $P = 0.09$).

### 4.3.2 Egg production

EGG WEIGHT AND DIMENSIONS

As explained in *Chapter 3*, the eggs within a clutch are not statistically independent (variation in egg size and weight was considerably greater between clutches than within, F-test, $p<0.00005$ in all cases) and, therefore, analyses of egg size between feeding treatments were carried out on clutch means. Table 4.6 shows mean egg length, width, volume and weight for the three treatment groups. None of the egg measurements differed significantly between treatments.

**Table 4.6** Mean (⊥ S.E. (n)) egg measurements for control and supplementary fed birds.

| | HIGH Q PROTEIN | LOW Q PROTEIN | CONTROL | Significance |
|---|---|---|---|---|
| Egg Length | 15.42± 0.13 | 15.45± 0.14 | 15.30± 0.14 | F= 0.33, d.f. =2,55, n.s. |
| (mm) | (20) | (18) | (20) | |
| Egg Width | 11.71± 0.07 | 11.73± 0.07 | 11.60± 0.08 | F= 0.87, d.f. =2,55, n.s. |
| (mm) | (20) | (18) | (20) | |
| Egg Volume | 1080± 20 | 1085± 19 | 1051± 19 | F= 0.93, d.f. =2,55, n.s. |
| ($mm^3$) | (20) | (18) | (20) | |
| Egg Weight | 1.139± 0.021 | 1.155± 0.021 | 1.113± 0.017 | F= 1.14, d.f. =2,55, n.s. |
| (g) | (20) | (18) | (20) | |

CLUTCH SIZE

Clutch size data was transformed by squaring, in order to normalise it, prior to performing parametric analysis. Clutch size declined significantly through the season. In order to control for this, an analysis of covariance was carried out with laying date as a covariate ($F_{reg.} = 26.36$, d.f. = 1,57, $P < 0.0005$; $F_{factor} = 4.15$, d.f. = 2,55, $P < 0.025$; $F_{model} = 13.01$, d.f. = 3,54, $p < 0.0005$). After controlling for laying date, there is no difference in mean clutch size between the treatment group receiving "low quality" protein and the control group, whilst the group receiving "high quality" protein shows a significantly greater mean clutch size than either of the other two groups; see Figure 4.1 and Table 4.7.

**Table 4.7**: Mean (± S.E. (n)) clutch size in control and supplementary fed birds; calculated using square transformed data. Values presented have been back-transformed.

| | HIGH Q PROTEIN | LOW Q PROTEIN | CONTROL |
|---|---|---|---|
| Clutch Size | 10.58 | 9.56 | 8.65 |
| | + 0.35, –0.37 | + 0.50, –0.56 | + 0.52, –0.60 |
| | (20) | (18) | (20) |
| Clutch Size Adjusted for Laying Date | 10.35 | 8.68 | 8.77 |

### 4.3.3 Incubation

Data on incubation is presented in Table 4.8. None of the incubation parameters examined differed significantly between treatment groups.

**Table 4.8**: Mean values (± S.E. (n)) for incubation parameters in control and supplementary fed birds.

| | HIGH Q PROTEIN | LOW Q PROTEIN | CONTROL | Significance |
|---|---|---|---|---|
| [a] Onset of | 15.95± 1.00 | 15.00± 1.06 | 17.24± 1.08 | F= 1.09, d.f. =2,48, |
| Incubation | (19) | (15) | (17) | n.s. |
| Duration of | 13.72± 0.59 | 13.36± 0.32 | 12.18± 0.52 | F= 2.13, d.f. =2,40, |
| Incubation (days) | (18) | (14) | (11) | n.s. |
| Delay in Onset of | −0.21± 0.71 | 0.53± 0.27 | 1.53± 0.65 | F= 2.17, d.f. =2,48, |
| Incubation (days) | (19) | (15) | (17) | n.s. |

[a] Mean dates in days from April 29$^{TH}$

### 4.3.4 Hatching parameters

Hatching data is presented in Table 4.9. None of the parameters examined differed significantly between treatment groups.

**Table 4.9**: Mean (± S.E. (n)) or median (interquartile ranges (n)) values for hatching parameters in control and supplementary fed birds.

| | HIGH Q PROTEIN | LOW Q PROTEIN | CONTROL | Significance |
|---|---|---|---|---|
| [a] Hatching Date | 29.89± 0.97 | 28.57± 1.14 | 29.09± 0.87 | F= 0.46, d.f. =2,40, n.s. |
| | (18) | (14) | (11) | |
| Hatching Success | 0.80 | 0.89 | 0.81 | $\chi^2$ = 0.43, d.f. = 2, |
| | 0.77 - 1.00 | 0.52 - 0.94 | 0.00 - 1.00 | |
| | (20) | (18) | (20) | n.s. |
| Hatching Asynchrony (days) | 1.50 | 1.50 | 2.50 | $\chi^2$ = 3.75, d.f. = 2, |
| | 1.50 - 2.50 | 1.50 - 1.75 | 1.50 - 2.50 | |
| | (18) | (14) | (11) | n.s. |

### 4.3.5 Chick parameters

Chick size measurements and weifgts are shown in Table 4.10. None of the parameters examined differed significantly between treatment groups.

**Table 4.10**: Mean (± S.E. (n)) values for brood size and chick measurements at hatching, 7 days and 14 days in control and supplementary fed birds.

| | HIGH Q PROTEIN | LOW Q PROTEIN | CONTROL | Significance |
|---|---|---|---|---|
| Hatchling Brood Size | 8.56± 0.47 (18) | 7.86± 0.43 (14) | 7.36± 0.88 (11) | F= 1.11, d.f. =2,40, n.s. |
| 7 Day Brood Size | 8.27± 0.60 (15) | 7.62± 0.40 (13) | 7.56± 0.90 (9) | F= 0.44, d.f. =2,34, n.s. |
| 14 Day Brood Size | 8.20± 0.60 (15) | 7.54± 0.39 (13) | 7.33± 0.91 (9) | F= 0.56, d.f. =2,34, n.s. |
| Hatchling Weight (g) | 1.01± 0.02 (18) | 1.07± 0.05 (14) | 0.97± 0.04 (11) | F= 1.62, d.f. =2,40, n.s. |
| 7 Day Chick Weight (g) | 7.32± 0.27 (15) | 7.39± 0.30 (13) | 7.25± 0.39 (9) | F= 0.05, d.f. =2,34, n.s. |
| 7 Day Chick Tarsus (mm) | 15.25± 0.34 (15) | 15.38± 0.37 (13) | 15.26± 0.43 (9) | F= 0.04, d.f. =2,34, n.s. |
| 14 Day Chick Weight (g) | 11.82± 0.15 (15) | 11.69± 0.18 (13) | 11.83± 0.26 (9) | F= 0.16, d.f. =2,34, n.s. |
| 14 Day Chick Tarsus (mm) | 19.96± 0.13 (15) | 19.97± 0.13 (13) | 20.09± 0.21 (9) | F= 0.19, d.f. =2,34, n.s. |
| 14 Day Chick Wing (mm) | 41.73± 0.73 (15) | 41.83± 0.80 (13) | 41.74± 1.00 (9) | F= 0.01, d.f. =2,34, n.s. |

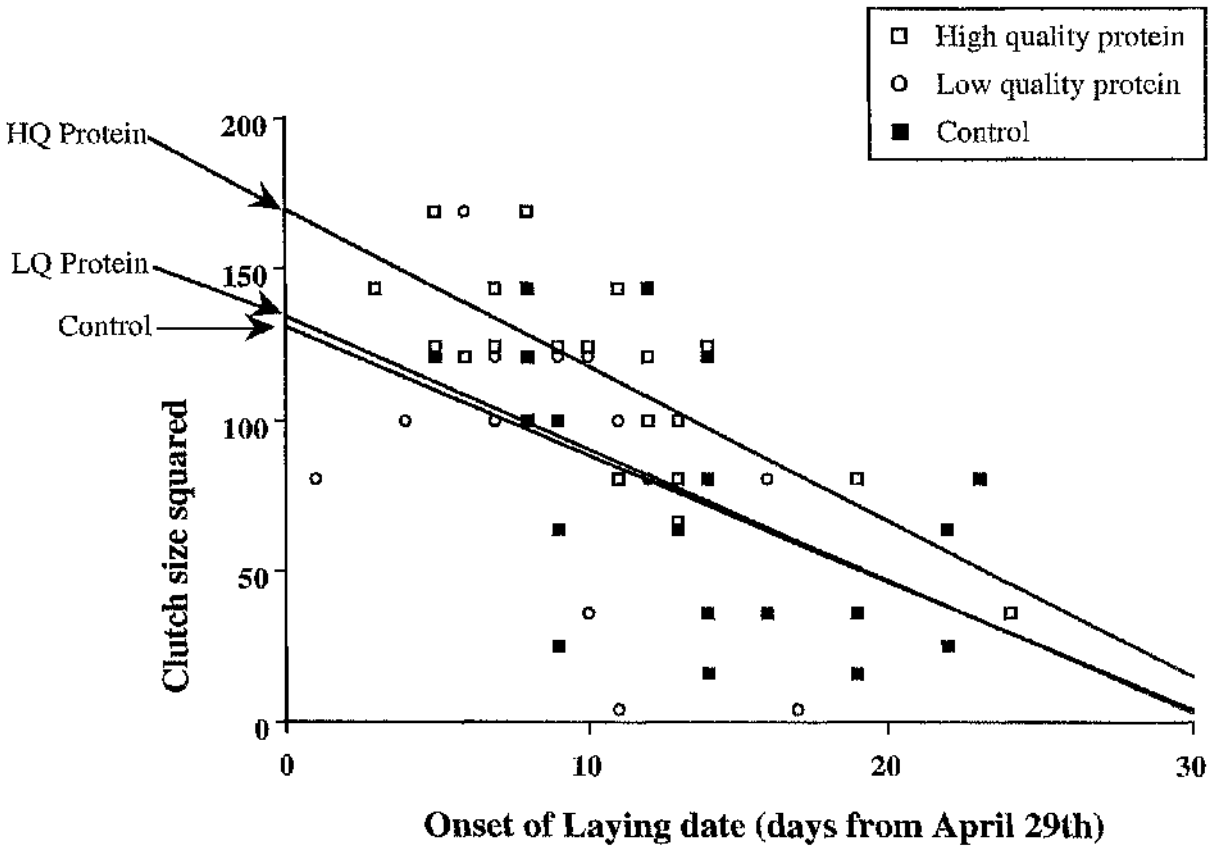



**Figure 4.1**: Regressions of clutch size against laying date for each experimental treatment group.

## 4.4 Discussion

### 4.4.1 Response to the provision of supplementary food

SUPPLEMENTARY FEEDING AND TIMING OF LAYING

The onset of laying in the two fed groups was earlier than in the control group, with a mean advancement of approximately 3 days. This is comparable with the advancement produced in other supplementary feeding studies involving blue tits; 5 and 6 days in different years in Clamens & Isenmann's (1989) study and 4 days and 6 days in Nilsson & Svensson's (1993a) and Nilsson's (1994) study; and is very similar to the degree of advancement evoked by supplementary feeding, in the same population, in the previous year (2.5 days, *Chapter 3*; Ramsay & Houston 1997 ). The advancement was not statistically significant in an Anova, however, despite a mean advancement slightly larger than the previous year. This was most probably as a result of a slightly smaller sample size and one particularly late laying individual in the 'High quality protein' group.

Interestingly, the mean onset of laying dates for the control groups in both years were virtually identical (8.5 and 8.4 days from April 29$^{TH}$ for 1994 and 1995 respectively). This, in conjunction with the very similar degree of advancement in the two years, supports the hypothesis that once food levels pass a threshold level, onset of laying is determined by other environmental factors such as daylength (*Chapter 2*; Ramsay & Houston 1997).

VARIATION IN EGG PRODUCTION AS A RESULT OF SUPPLEMENTARY FEEDING

Mean egg size was slightly larger in the two fed treatment groups compared with control but this difference was far from significant. The mean size of eggs in the control group was similar to that for the control group in the previous year - if anything control eggs were slightly smaller than in 1994. Therefore, in terms of anatomical / physiological ability, birds in the fed treatment groups should have had the 'scope' to increase the size of their eggs. The fact that they didn't suggests either that the supplementary diet they received did not provide enough of the limiting nutrient(s) to allow an increase in egg size or as a result of other influential environmental factors the birds were unable to, or "chose" not to, increase egg size. I consider now whether the lack of an increase in egg size in the group receiving the 'high quality protein' supplementary food could have been the result of a remaining nutritional constraint.

## A NUTRITIONAL DEFICIENCY IN THE 'HIGH QUALITY PROTEIN' SUPPLEMENTARY DIET?

The 'high quality' diet contained approximately the same levels of the assumed five potentially limiting amino acids as whole hens' eggs (in terms of mg amino acid / g Nitrogen) and, consequently, approximately the same or slightly higher levels than were present in the egg-based supplementary food the previous year. The lack of an increase in egg size amongst those birds receiving the 'High quality' diet, whilst those receiving the egg-based diet the previous year showed a significant increase in egg size cannot, therefore, be explained in terms of composition of these amino acids in the supplementary diets. Amino acid intake from the supplementary diets may be influenced by more than just their amino acid composition. Many poultry can adjust their feed consumption to obtain adequate energy when receiving diets of varying energy content (Scott, Nesheim & Young 1982). It is, therefore, necessary to adjust the protein content of the diet, in relation to the energy level of the diet, so that the hen receives the appropriate amount of protein. We do not know how the blue tit determines its level of food intake, therefore it is possible that such a mechanism may operate. The energy : protein ratio in the egg-based diet is lower than for the 'high quality' diet (approx. 90% of the 'high quality' ratio). Therefore, *if* blue tits consumed supplementary food on the basis of energy content, the birds receiving the egg-based supplementary diet would consume more protein than those receiving the 'high quality' diet (approx. 10% more protein). However, this is crude protein and we are more interested in the five specifically supplemented amino acids. If we consider the energy : specific amino acid ratio between diets (for these five) we find that they are almost identical (ratio for egg-based diet is approx. 102 % of that for 'high quality' diet). This means that birds receiving the 'high quality protein' diet would consume about the same, or even slightly more, of these specific amino acids than those receiving the egg-based diet. Differences in energy : amino acid ratio cannot, therefore, explain the lack of an effect on egg size in the group receiving the 'high quality' supplementary diet. Another, similar, aspect of the supplementary diets to consider is nutrient density (i.e. nutrients per unit mass/volume). Nutrient density (both energy and protein) is greater in the 'high quality' diet than in the egg-based diet. Therefore, if blue tits were constrained in the amount of food they could physically ingest, the birds feeding on the 'high quality' diet should be able to consume more nutrients (including more amino acids) than those receiving the egg-based diet. Nutrient density also cannot, therefore, explain the lack of an increase in egg size either.

Having examined amino acid related differences in supplementary diets as an explanation for the differing effect on egg size, I now consider whether there are any other

nutritional explanations. Other potential candidates are vitamins, minerals and 'essential' fatty acids.

Peanuts, which formed the base for the 'high quality' diet, are deficient in Vitamin A and Vitamin D (Holland *et al* 1991); both of which (but especially Vitamin A) are present in fairly high levels in egg yolk (Holland *et al* 1991). Vitamin A is involved in development of the embryo and hatchability of eggs, but there is no evidence in poultry for any effect on egg size (Scott, Nesheim & Young 1982). Vitamin D plays a role in effective eggshell formation; being involved in the absorption of calcium from the intestinal tract; but again there is no evidence reported for any effect on egg size (Scott, Nesheim & Young 1982). Therefore, if there were any vitamin deficiency it would most probably also manifest itself in other symptoms, such as reduced hatchability or defective eggshells (Scott, Nesheim & Young 1982). There was no evidence for this. Further, the supplementary diets are, as the term implies, a supplement to the natural diet. Since vitamins are required in very small quantities, it is most likely that any that were deficient in the peanut-based diet would be obtained from the portion of the diet that was of natural origin. Finally, vitamin levels in the egg-based diet were probably reduced by the heating involved in preparation of the diet; thus narrowing the difference between supplementary diets. It is, therefore, very unlikely that any vitamin deficiency can explain the lack of an increase in egg size.

'Essential' fatty acids are another component of nutrition that is often overlooked. In poultry at least, linoleic and arachidonic acids are the main 'essential' fatty acids. Linoleic acid appears to have a marked effect upon egg size, and it is necessary to ensure that a sufficient amount of linoleic acid is included in the diet of laying hens to enable them to lay eggs of maximum size (Scott, Nesheim & Young 1982). Linoleic acid, however, is present in hens' eggs at only about 1.0g / 100g fresh weight, whilst peanuts and lard contain about 13.5g / 100g and 8.2g / 100g respectively(Holland *et al* 1991). Essential fatty acids would not, therefore, be limiting to birds receiving the peanut-based diets.

Finally, the first mineral to become limiting to a laying blue tit would probably be calcium. However, this potential constraint has already been examined and eliminated - see *Chapter 5*).

Having considered the points above, it appears, perhaps, more likely that the difference in observed effect on egg size between the group receiving the egg-based diet in 1994 and the group receiving the 'high quality' diet in 1995 was a result of the interaction of other environmental factors, rather than a nutritional deficiency in the 'high quality' diet.

Clutch size, however, was significantly affected by the provision of supplementary food. The group receiving 'low quality protein' had a mean clutch size slightly, but not significantly, greater than control whilst the group receiving 'high quality protein' had a

significantly larger mean clutch size than control. However, clutch size also fell through the season, thus introducing additional variation into the data. When this was taken into account, in an analysis of covariance, it could be seen that: firstly, all three groups showed parallel declines in clutch size through the season; secondly, that the slight increase in mean clutch observed in the group receiving low quality protein was entirely due to the slight advancement of laying date; and thirdly, that mean clutch size in the group receiving high quality protein was significantly greater than that in both the control group and the group receiving low quality protein and that this difference was consistent throughout the season. Mean clutch size, adjusted for laying date, in the high quality protein group was approximately 1.6 eggs greater than in the low quality protein or control groups.

Why did birds increase their clutch size in response to the 'high quality protein' supplementary food, but not their egg size, and why did clutch size still decline in parallel through the season? I cannot find a clear explanation. However, I shall now examine the information available regarding the influence of food supply on clutch size in the blue tit, and attempt to interpret the results of this study within the existing framework of knowledge.

### 4.4.2 The Influence of Food Supply on Clutch Size in the Blue Tit

There is evidence that in tits and other small, insectivorous passerines, clutch size is often related to natural food abundance (Järvinen 1982; Järvinen & Väisänen 1984; Perrins 1991). It is possible, however, that food supply is not the cause in such a relationship but that both parameters, in fact, vary in response to a third, environmental parameter. More conclusive evidence, perhaps, would come from supplementary feeding experiments and a number of such studies have been carried out on tits and other small passerines (see reviews in Martin 1987; Boutin 1990). The results of these studies, however, are mixed. Although most produce an advancement of laying date, only a few have resulted in any increase in clutch size (Nager, Rüegger & van Noordwijk, in press; Arcese & Smith 1988; Hogstedt 1981). This would suggest that clutch size is frequently not determined by food supply (i.e. it is determined by other factors) and/or the supplementary food used does not adequately mimic an increase in natural food supply.

In cases where the provision of supplementary food does result in an increase in clutch size, there are two main alternative mechanisms by which this could occur. Firstly, clutch size could be directly constrained by the availability of nutrients to form more eggs (food supply at time of laying is *proximate* and *ultimate* constraint on clutch size). In this scenario, supplementary food would allow the female to form the size of clutch she 'desires' - as determined by other factors. Alternatively, females may adjust their clutch size in response to current food supply if this provides an indication of food levels at some future, potentially food limited, stage of the breeding cycle such as chick rearing (food supply at time of laying is only *proximate* constraint, whilst food supply at future stage of breeding is *ultimate* constraint). I shall now consider the evidence for these two alternative explanations.

#### DOES FOOD SUPPLY AT THE TIME OF EGG LAYING DIRECTLY CONSTRAIN CLUTCH SIZE ?

Female blue tits lay the largest clutches of any known altricial bird, with a mean of around 11 eggs in many areas of central and northern Europe and complete clutches are often heavier than the laying female's own body weight. Thus, endogenous reserves could only provide a fraction of the total nutrients required for formation of the clutch and females must, therefore, rely either wholly or predominantly on dietary intake. This was confirmed in a recent study by Woodburn (1997).

The cost of producing the first egg in a clutch has been shown to be higher than producing the later ones, due to concomitant growth of the oviduct (Krementz & Ankney 1986; Meijer, Masman & Daan 1989). In addition, it has been reported that food availability generally improves on a day to day basis at this time (Perrins 1970; Daan *et al* 1988). Therefore, if a female were able to obtain sufficient nutrients exogenously to form an egg at the start of a clutch, there is no obvious reason why she would not be able to go on obtaining nutrients at this rate and produce a clutch as large as she 'desired'.

If clutch size *were* to be constrained by food supply, females would have to be relying, in part at least, on endogenous reserves for egg production. If the diet did not provide enough nutrients each day to form an egg, and the female had to augment the dietary intake with nutrients drawn from endogenous reserves, then these endogenous reserves would gradually deplete and if they ran out or reached a critical threshold level, the female would be unable to form any more eggs and the clutch size would be determined. If this happened before the 'desired' clutch size was reached (as influenced by other factors) the clutch size would, by definition, be constrained by food supply. However, as mentioned earlier, natural food abundance may increase on a daily basis at this time. Therefore, even if endogenous reserves were required to augment dietary intake at the beginning of the clutch, unless this requirement was fairly large, after laying a few eggs the natural food abundance may have increased sufficiently to obviate any subsequent requirement for endogenous reserves. Woodburn (1997) found that body protein levels did not fall through the laying period and fat levels fell by only 0.018g per day (a small fraction of the birds daily energy requirements at this time). It seems very unlikely that increasing natural food levels would not usually become sufficient to outweigh such a small requirement. Further evidence that clutch size in tits may not be directly constrained by food supply has come from egg removal experiments. Both Winkel (1970) and Haywood (1993) report that if eggs are removed from the nests of laying blue tits, they can be induced to produce significantly more eggs than unmanipulated controls. Finally, and very importantly, is the issue of 'courtship' feeding by the male. Males of some tit species provision their partners with an increasing amount of food through the pre-laying, laying and incubation periods (Nilsson & Smith 1988). It has been estimated that in blue tits, during the laying period, provisioning of the female by the male provides approximately enough food for the production of each egg (Royama 1966; Krebs 1970).

Consider, now, our supplementary feeding experiment. Both experimental diets had identical energy and crude protein levels, and differed only in amino acid composition. Only the diet containing 'high quality' protein, however, resulted in an increase in clutch size. Therefore, if clutch size were directly constrained by some aspect of food supply, it could not be energy supply. Rather, it would have to be the supply of 'high quality' protein (i.e. specific

amino acids). However, there was no clear evidence in Woodburn's study (1997) for any detectable use of endogenous protein reserves in female blue tits (as detailed earlier, this is a requirement for a direct nutritional constraint on clutch size to exist). However, it may be that endogenous reserves are not used for substantial amounts of protein, but to supply only small amounts of limiting amino acids, and in this case changes in muscle mass may not be substantial (Selman & Houston 1996).

Finally, to paraphrase Martin (1987), nutritional constraints on egg production should perhaps first be expressed by reduced egg size rather than egg number, because changes in clutch size represent major changes in reproductive potential. With decreasing natural food levels, this would continue until further reductions in egg size would result in an increase in mortality probabilities of the young such that a reduction in clutch size would better optimise reproductive success. Thus, if food directly limits egg production, egg size should probably increase with increasing food abundance. However, in our feeding experiment, neither supplementary diet resulted in an increase in mean egg size.

## FOOD SUPPLY AT THE TIME OF EGG LAYING AS A CUE DETERMINING CLUTCH SIZE

From the evidence presented above, it appears perhaps unlikely that clutch size in blue tits would normally be directly constrained by food supply. The alternative explanation is that, when food supply does influence clutch size (and we know it does - see earlier), it does so by providing information about the probable future. Some aspect of current food supply may be used as an indication of food abundance at some future, food limiting, stage of the breeding cycle. Females could then adjust the size of their clutch in response to anticipated future food levels and their own foraging ability / quality, so as to maximise their reproductive fitness. It is often stated that, in situations such as these, females adjust their clutch size according to their perceived ability to feed and rear young during the nestling stage (bearing in mind any reproductive costs associated with increased reproductive effort), so as to maximise fitness/reproductive output (Lack 1947, 1966; Perrins & Moss 1975; Slagsvold 1982; Hogstedt 1980; Andersson 1976). There is some evidence for other species, that the clutch size a female lays is, actually, the optimal size for her to rear and, therefore, maximises personal fitness. In experimental manipulations, enlarging or reducing the brood size often results in reduced numbers of recruits (magpie *Pica pica*, Högstedt 1980; collared flycatcher *Ficedula albicollis*, Gustafsson & Sutherland 1988; great tit *Parus major*, Pettifor, Perrins & McCleery 1988; and pied flycatcher *Ficedula hypoleuca*, Alatalo & Lundberg 1989) or sufficiently reduced parental

survival and/or future reproductive success to outweigh any increase in current fledgling production. Nur (1984a,b) found that in blue tits, female body weight and survival and offspring body weight generally declined with increasing brood size. However, this effect occurred irrespective of whether females were rearing their natural, intended brood size or one which had been artificially increased or reduced (Nur 1986). Also, the optimal brood size (in terms of numbers of recruits) in any one year was similar for all individuals and was greater than the natural mean clutch size (Nur 1986). Nur's work, therefore, provides virtually no evidence that individual blue tits adjust their clutches according to their ability to rear nestlings.

How can we explain the apparent discrepancy between the observed brood sizes and the apparently optimal brood sizes? If we assume for the present that clutch size /brood size is not determined by direct nutritional constraints at the time of egg laying, and female blue tits appear to be capable of rearing larger broods than they normally produce, the only other potentially limiting stage of the breeding cycle is incubation. Is there any evidence that incubation is food limited and/or that clutch size is adjusted to the female's ability to incubate the eggs. Haftorn & Reinertsen (1985) found that the energetic cost of simply maintaining incubation temperature in the blue tit increased with clutch size, below 16°C. Further, the energetic cost of maintaining incubation temperature was fairly high: 50 - 90% as much as basal metabolic rate. Smith (1989), manipulating the clutch size of blue tits at the beginning of the incubation period found that a mean clutch size of 14.3 eggs took on average, approximately 12 hours longer to incubate than a mean clutch of 7.8. Although this does indicate that larger clutches take longer to incubate, and it is known that time saving is important to breeding blue tits (*Chapter 3, discussion*), it is difficult to imagine that such a large reduction in clutch size could be compensated for by a saving of only 12 hours. Slightly better evidence, for a constraint on incubation, comes from Nilsson & Smith's (1988a) study on Marsh tits (a species similar in many ways to the blue tit) in which the level of male 'courtship feeding' was shown to increase substantially from the laying period to the incubation period, and the length of the incubation period was significantly negatively correlated with the rate of provisioning of the female by the male. Differences in observed rates of male provisioning could affect the overall length of the incubation period by greater than 3 days. The significance of male involvement is further supported by the finding (in Nur's (1986) brood manipulation studies) that the weight of male blue tits was negatively related to their original clutch size; perhaps indicating a cost of increased provisioning of the female. Supplementary feeding of female blue tits has also been shown to shorten the incubation period and increase hatching success, although this was only by 18 hours and from 92% to 98% respectively (Nilsson & Smith 1988b). Thus, there is some evidence that length and efficiency of incubation can be

affected by food supply and clutch size but it is not clear whether this effect could ever be strong enough to constrain clutch sizes to those that we observe.

An alternative hypothesis has been proposed by Nur (1984b). In a model to determine optimal brood size (incorporating $m_i$ : number of offspring surviving to breed from brood size i, and $p_i$ :annual survival rate of adults rearing a brood of size i), it was found that predicted optimal brood size matched apparent optimal brood size from brood size manipulation experiments (Nur 1984a,b), *when the values used for* $m_i$ *and* $p_i$ *were treated as constants through time*. However, this assumption is not true and the model predicts that a large variance in $m_i$ could favour individuals rearing smaller broods if some years prove catastrophic. As Nur (1984b) points out "individuals consistently rearing small broods will be favoured over those rearing large broods because the former will more likely survive to the next year and breed when conditions are better".

Unpredictability could also favour smaller clutches for a slightly different reason. Weather changes and temperature fluctuations are not uncommon in temperate areas and it is known that a sudden rise or fall in temperature can dramatically alter the development and consequently the availability of caterpillars (Varley 1970; Perrins 1973, 1979). Similarly, a sustained period of heavy rain or strong winds can seriously impair a blue tit's foraging ability. If adults rearing an apparently optimum brood size (for the general environmental conditions and individual 'quality') were foraging and feeding young at close to their maximum/optimum capacity and the nestlings were at some optimum mass (as a result of trade-off between nestling mass and brood size) when adverse conditions struck, such adults may be unable to increase foraging effort sufficiently and/or nestlings may have insufficient body reserves, to prevent total brood loss. A female rearing an apparently smaller than optimal brood size, however, may be better able to cope with such environmental variability.

Boyce & Perrins (1987) present evidence for great tits, that year-to-year environmental variability best explains why average clutch size is below the apparently most productive clutch size. "Years that are poor for survival of young *appear to* affect individuals laying larger clutches much more than they affect those laying smaller clutches *and, as a result*, it is more advantageous, in the long term, for birds to lay clutches smaller than the most productive clutch size" (words in italics are mine).

Thus, females may indeed adjust clutch size in response to own individual 'quality' and anticipated food levels at time of chick rearing (or perhaps incubation) **but** adjust it to a level slightly below the apparent optimum as a result of variability in $m_i$ and unpredictable environmental factors.

It would appear that, in terms of clutch size determination, female blue tits may respond to food supply at the time of laying as a cue; presumably providing information about some aspect of food abundance at a future stage of the breeding cycle. In our supplementary feeding experiment, females receiving the 'high quality protein' diet significantly increased their mean clutch size, whilst those receiving the 'low quality protein' diet produced a mean clutch size which did not differ significantly from control. Thus, females did not increase clutch size in response to general food abundance, or to energy supply/intake , or to crude protein supply/intake: all these qualities were identical between experimental diets. Rather, females adjusted their clutch sizes in response to either the quality of the food supply (in terms of amino acid composition) *per se*, or to the supply/intake of food of a particular quality (in terms of amino acid composition). This, therefore, suggests that the amino acid composition of the diet at the time of laying may convey some information to the female about future food abundance or perhaps future food quality. How could this operate?

It is difficult to say at this stage of investigations into food quality, exactly how this might operate and what information protein quality/amino acid composition might relay to the laying female. However, I offer here a few potential hypotheses:

1) At the time of laying, female blue tits may consume a considerable amount of plant material (primarily arboreal bud tissue), in addition to invertebrate prey (Betts 1955). Although the female probably gains some nutrients from this plant material, it may largely be consumed 'incidentally' whilst foraging for bud-inhabiting larvae. The nutritional value of bud tissue is probably fairly low and, in particular, it's protein is likely to be of 'low quality', in comparison to animal protein, in terms of requirements for egg production (eg. plant material generally has relatively low levels of methionine and lysine; Scott *et al* 1982). It may be that, as caterpillar density increases, the frequency of buds containing caterpillars, and the number of caterpillars per bud increases. If this were the case, then the female may ingest a lower proportion of plant material : invertebrate material in years of higher caterpillar abundance. Therefore, a higher proportion of amino acids such as methionine or lysine in the diet may indicate to the female that caterpillar abundance is high.

2) The diet of the laying female is generally composed of a variety of different invertebrates (a proportion of which is caterpillars) and some vegetable matter, as mentioned above. The young, however, are fed almost exclusively on caterpillars and, therefore, it is primarily the abundance of caterpillars (rather than food/invertebrate supply in general) at the time of laying which is important for forecasting future prey abundance for raising young. If caterpillars have an amino acid composition which is noticeably different to that of other invertebrates, female blue tits

may be able to use this (perhaps in conjunction with visual cues) to estimate caterpillar abundance.

## OTHER NON-NUTRITIVE FACTORS INFLUENCING CLUTCH SIZE

Although food supply does have an important influence on clutch size, a number of other factors are also known to exert a major influence.

In blue tits and other Parids, clutch size is often reported to decline through the breeding season (eg. Verhulst & Tinbergen 1991; Nilsson & Svensson 1993a,b) and a similar result was found in this study. As detailed earlier (*Chapter 3-discussion*), time savings are very important in determining the breeding success of tits, and this may also have an influence on clutch size. Clutch size may decrease through the season due to a tactical decision by the female, based on when she is either able to, or 'decides' to begin laying. The survival (von Haartmann 1966; Perrins 1979; Newton & Marquiss 1984; Norris 1993) and prospects of establishment and breeding success (Nilsson & Smith 1988; Nilsson 1989, 1990; Hochachka 1990) of young declines the later in the season they hatch/fledge. Therefore, eggs will be of declining value as the season progresses (Daan *et al* 1988). Females may increase the overall value of later clutches by reducing clutch size, since incubation and hatching are postponed by one day for each additional egg laid (Drent & Daan 1980; Nilsson & Svensson 1993a). Additionally, as available food levels decline and become more unpredictable towards the end of the breeding season (Perrins 1965), females may be unable to raise a large brood. Also, females should be able to raise larger young by reducing the number they have to feed. Although probably not as important as timing, fledgling mass and recruitment rate are correlated. (Perrins 1965; Tinbergen & Boerlijst 1990; Linden *et al* 1992).

Tits also tend to lay smaller clutches in years when breeding density is high (Perrins 1965). This may be as a result of competition for food amongst fledged young. Kluijver (1971) demonstrated that the survival of fledgling Great tits was markedly affected by the numbers of other young Great tits present.

## THE EFFECT OF EXPERIMENTALLY INCREASED CLUTCH SIZE

Mean hatchling brood size, 7 day brood size and 14 day brood size were all larger in the group receiving high quality protein although this difference was not statistically significant. The difference in mean brood size between the group receiving high quality protein and controls was approx. 0.8 chicks and 0.9 chicks for hatchlings and fledglings respectively. Therefore,

although not statistically significant, there was a suggestion that the experimentally increased clutches did go on to produce more young. Why was there a significant difference between groups in mean clutch size and not in mean brood size? This could simply be a statistical problem - as a result of reduced sample sizes or it may have an ecological explanation. If females increased their clutches in response to additional food, then they may have increased them above the level at which they could successfully raise all the resultant chicks when the additional food was removed.

We can conclude that dietary protein quality may be an important factor in the proximate control of reproductive output in blue tits. At this stage of investigations, however, it remains unclear to what extent dietary protein quality acts as either a direct nutritional constraint on egg production or a cue indicating future food quality or availability.

# CHAPTER 5

## Does acid rain and calcium supply limit eggshell formation for blue tits in the UK?

*"One thing I have learned in a long life: that all our science, measured against reality, is primitive and childlike - and yet it is the most precious thing we have."*

Albert Einstein

## 5.1 Introduction

Birds require large amounts of calcium for effective eggshell formation (Robbins 1993; Graveland 1995). Some large species are able to mobilise skeletal calcium for this purpose, but in small passerines, this can provide only a small fraction of that required for the clutch (Ankney & Scott 1980; Graveland & VanGijzen 1994; Schifferli 1979; Houston *et al* 1995a; Graveland & Berends 1997). In species laying large clutches, the calcium content of the eggs can approach, or exceed, that of the female's entire skeleton (Maclean 1974; Perrins 1979; Jones 1976; Ormerod *et al* 1988). Most small birds, therefore, must depend upon obtaining sufficient calcium from their diet.

The calcium content of usual prey items, such as insects and spiders or seeds and fruits, is generally insufficient for eggshell production (Studier & Sevick 1992; Maclean 1974; Krapu & Swanson 1975; Turner 1982; Graveland & vanGijzen 1994). To overcome this, many birds have been shown to forage actively for calcium-rich items during the egg production period, such as small pieces of bone, calcareous rock or pieces of eggshell (Sadler 1961; Repasky *et al* 1991; Jones 1976: Maclean 1974; Kopischke 1966; Krementz & Ankney 1995; Nisbet 1997; Brenninkmeijer, Klaassen & Stienen 1997). The most important natural source for temperate passerines such as tits, however, appears to be snail shells (Ankney & Scott 1980; Shifferli 1977; Creutz 1953).

Interest in this subject has increased recently due to the influence of acid rain. Anthropogenic sulphur dioxide ($SO_2$), oxides of nitrogen ($NO_x$) and ammonium ($NH_3$) emitted into the atmosphere, can subsequently be converted into sulphuric and nitric acid in the atmosphere or soil; as such, they greatly increase the rate and extent of natural soil acidification. Increased soil acidity can result in enhanced leaching of calcium and affected areas often contain reduced numbers of snails and other calcium-rich items (Graveland 1990; Scheuhammer 1991; St. Louis & Breebaart 1991; Graveland *et al* 1994; Graveland & van der Wal 1996). An increase in soil acidity can also promote the mobilisation of aluminium and heavy metals (Cronan 1994; Merino & GarciaRodeja 1997). Reduced soil calcium concentrations, coupled with elevated aluminium levels, can severely impair the uptake of calcium by plants. As a result, the calcium content of usual dietary items such as vegetation or herbivorous arthropods also declines, further exacerbating the calcium supply problem (Drent & Waldendorp 1989).

In response to this, several studies in areas of high acid rain have shown an increase in the proportion of eggs laid with no shell at all or with shells of poor quality (thin and porous) which have greatly reduced hatching success (Drent & Waldendorp 1989; Graveland *et al*

1994; Graveland & van der Wal 1996; Ormerod *et al* 1988; Blancher & McNichol 1988). The association of calcium deficiency with these eggshell defects was confirmed in an experiment with great tits (*Parus major*) in the Buunderkamp forest in the Netherlands where the provision of calcium-rich items at the nest significantly reduced the incidence of eggshell defects (Graveland *et al* 1994). In contrast, in an area of the American west relatively unaffected by acid precipitation, the provision of supplementary calcium had no significant effect on the eggs of breeding house Wrens, *Troglodytes aedon* (Johnson & Barclay 1996).

In studies of blue tits (*Parus caeruleus*) around Loch Lomond I have shown that nutrient availability can limit egg production (Ramsay & Houston 1997; *Chapter 3 & 4*). In this chapter I consider whether calcium availability may also limit egg formation at this site. Calcium supply might be expected to be limiting for two reasons. Firstly, calcium levels in the environment vary substantially depending on local geology, and the basal rocks of the Scottish Highlands have among the lowest levels found in Britain. Several studies report that snail density, and number of snail species, are strongly positively correlated with the calcium content of the soil or litter (Burch 1955; Wäreborn 1969, 1970; Graveland 1995;). Our study area in west-central Scotland had an exchangable soil calcium level of only 0.02 mg/g (Langan, *pers. comm*); half the value found at the Buunderkamp forest in the Netherlands (0.04 mg/g, Graveland, *pers. comm*). Secondly, low natural calcium availability will be exacerbated by acid rain. West central Scotland experiences the highest levels of acid rain in Britain (>0.06g $H^+$ $m^{-2}$ $yr^{-1}$, CLAG 1994). The potential impact of acid deposition in any area is summarised by the term 'critical load', which defines the threshold level above which long term damage or significant harmful effects will occur. The poorly buffered soils of the Scottish Highlands, north of the Highland Boundary Fault, are extremely sensitive to acidification, exhibiting some of the lowest critical loads in the UK (0.5 keq $H^+$ $ha^{-1}$ $yr^{-1}$, CLAG 1994). Consequently, critical loads are exceeded over much of the Highlands, with the greatest exceedences occurring in west central Scotland (exceedence >1.0 keq $H^+$ $ha^{-1}$ $yr^{-1}$, CLAG 1994). The critical load for acidity in the Buunderkamp forest was approximately 1.5 keq $H^+$ $ha^{-1}$ $yr^{-1}$, three times less sensitive than the Scottish Highlands, and the exceedence approximately 3.5 keq $H^+$ $ha^{-1}$ $yr^{-1}$ (de Vries, pers comm.).

One might, therefore, expect that tits breeding in this area of the Scottish Highlands would face even greater difficulties in obtaining calcium for eggshell formation than the birds in the Buunderkamp region in the Netherlands. I here report on the effects of a calcium supplementation study, and also consider the abundance of calcium sources for birds in this area.

## 5.2 Methods

### 5.2.1 Supplementary feeding

This study was carried out in 1994 on populations of blue tits utilising nestboxes (wooden and concrete) situated in mixed oak woodland around Tarbet and the RSPB reserve at Inversnaid on the perimeter of Loch Lomond in west-central Scotland.

Nestboxes were first checked in mid-April, and then weekly until egg laying was completed. When a new nest was found, which was at least one quarter built, it was assigned randomly to either the calcium-supplemented group or to the control group.

Supplementary calcium was provided by hanging a dry, weighed cuttlefish bone close to the nestbox and placing two plastic trays, containing 200g of oyster grit and 200g of crushed eggshell, on the ground near the base of the tree. Blue tits are highly territorial at this time and, therefore, the supplementary calcium should only be available to the experimental pairs.

All eggs were numbered, using permanent OHP pens, weighed (to 0.05g) with a 5g pesola spring balance and the length and width measured (to 0.05mm) with vernier callipers. Egg volume was calculated using the equation: Egg Volume = 0.51× egg length × egg width$^2$ (from Hoyt 1979).

In addition, one egg per nest was removed for analysis of eggshell weight/thickness. These eggs, once removed, were placed in individually labelled containers and stored in a refrigerator until analysed. In order to facilitate separation of contents from shell, eggs were cooked in an oven at 100° C for 1 hour; having first made a small hole at the airspace to allow expanding gas to escape. After separation, all membrane was removed from the inner surface of separated eggshells, before they were dried in an oven, at 110° C for 2 days, and weighed. A shell thickness index was calculated by dividing shell weight by an index of shell area (egg length × egg width).

Approximately 7 weeks after the end of laying, all nests were checked for unhatched eggs in order to determine hatching success.

### 5.2.2 Estimating snail density

Snail density was determined by a similar methodology to that described by Graveland (1995) in which randomly selected quadrats of 1m x 1m had all loose leaf litter and the top 2cm of mineral soil removed. These were taken to the laboratory and each sample searched manually,

sometimes with the aid of sieves to fractionate samples, for 2 man hours per sample. This work was carried out by Zoology degree students from Glasgow University.

## 5.3 Results

### 5.3.1 Calcium supplementation experiment

The results from Tarbet and Inversnaid were very similar so the data have been combined.

**Table 5.1:** Mean values ( ± S.E. (n) ) of reproductive parameters for pairs of blue tits provided with supplementary calcium and for unsupplemented controls.

| Parameter | Control | Calcium-supplemented | Statistics [a] |
|---|---|---|---|
| Egg weight (g) | 1.14 ± 0.02 (15) | 1.12 ± 0.02 (12) | $t_{25} = 0.65$, n.s. |
| Egg volume ($mm^3$) | 1082 ± 18 (15) | 1075 ± 19 (12) | $t_{25} = 0.27$, n.s. |
| Clutch size | 9.53 ± 0.74 (15) | 9.33 ± 0.61 (12) | $t_{25} = 0.20$, n.s. |
| Onset of laying (days from April 30th) | 10.47 ± 1.20 (15) | 9.42 ± 1.20 (12) | U = 79, n.s. |
| Shell weight (g) | 0.070 ± 0.001 (13) | 0.069 ± 0.002 (8) | $t_{18} = 0.56$, n.s. |
| Shell thickness index ($g\ mm^{-2}$) | 0.3865 ± 0.006 (13) | 0.3828 ± 0.008 (8) | $t_{18} = 0.37$, n.s. |
| Hatching success | 1.00 (median) (15) | 1.00 (median) (12) | U = 80, n.s. |

a : All tests are two-tailed.

Table 1 shows that supplementary calcium did not result in any significant increase in egg weight or size, nor did it produce any increase in shell weight or thickness. Similarly, there was no significant difference in clutch size between the groups. The mean onset of laying was slightly earlier in the calcium supplemented group (approx. 1 day) but this difference was far from significant. Hatching success, expressed as the proportion of all eggs in a clutch which hatched, was also not significantly different between treatment groups.

### 5.3.2 Snail density

Large snails were completely absent from the study area, and careful examination of the leaf litter revealed only two gastropod species: *Carychium minimum* (Mueller) and *Discus rotundatus* (Mueller). 22 quadrats, of $1m^2$, were found to contain only 8 shells (all of *D.rotundatus*, mean diameter ( ± S.E.), 3.20mm ± 0.13mm), despite intensive searching (approx. 5 man hours per snail!).

In 1995, as part of an ongoing study into the effects of food supply and quality on reproduction in blue tits, a female blue tit was captured on the nest, in early evening, during the egg laying period. An examination of the gizzard contents revealed a large number of small snails and a number of other calcareous fragments, including a small bone and a tooth. The snails were identified as juvenile examples of *C. minimum* and *D. rotundatus*, confirming that these snails are the calcium source used by tits in this area. There were 21 *C. minimum* (1.6-1.9 × 0.7-0.9mm) and 9 *D. rotundatus* (1.1-4.4 × 0.6-1.7mm) present, with a combined dry mass of 5.2mg and 62.3mg respectively. The total dry mass of calcareous items was 76.4mg.

## 5.4 Discussion

The provision of supplementary calcium to breeding pairs of blue tits did not result in a significant enhancement of any of the reproductive parameters examined. Why?

No enhancement would be detected if the supplementary calcium was provided in an unsuitable form for the females to use. This is unlikely since other birds (including tits) have effectively used similar forms of calcium-rich material (Jones 1976; Repasky *et al* 1991; Graveland *et al* 1994; Krementz & Ankney 1995) and although no systematic observations were carried out, females in our study were seen pecking at the dishes of grit and eggshell.

Alternatively, control females could have gained access to the supplementary calcium at the experimental nestboxes. Again, this is unlikely because blue tits are highly territorial and supplementary calcium was not provided at a nestbox until a pair had become established and nestbuilding was almost complete.

The most likely explanation, therefore, for the observed result is that the control females were able to obtain sufficient calcium from natural sources. This explanation gains some support from an examination of the gizzard contents of a female blue tit sampled in early evening during the laying period, which contained 76.4 mg of calcareous material, 67.5mg of which was snail shell. This compares with a mean value of 62mg of snail shell found in the gizzards of great tits in an area of the Netherlands where defective eggs were uncommon (Graveland, pers. comm.). Blue tit eggs have a mean dry shell mass approximately $^2/_3$ that of great tits, therefore the mass of calcareous material found in the gizzard perhaps indicates that adequate amounts were being obtained for effective eggshell formation in a blue tit. Such findings, however, can provide only an indication of the calcareous items being obtained. During egg laying, captive great tits have been observed to consume approx. 65mg of calcium per day, in the form of snail shells (Graveland 1995); equivalent to approx. 197mg dry weight of snail shell. Apparently then, the amount present in the gizzard shortly after going to roost may represent only a proportion of the total days consumption of calcium-rich items. Assuming a calcium content of 33% for snail shell and 35 % for eggshell, and a digestion and deposition efficiency of 60% (Graveland 1995), the mass of calcareous items found in the blue tit's gizzard would only provide approx. 62% of required daily dietary calcium. Much of the snail shell may already have been broken down into very small fragments, passed into the stomach and/or been digested and therefore not detected. In addition, around 10% of daily calcium requirements may be obtained from the usual invertebrate prey taken (Graveland 1995).

From the number of snails found in the gizzard one might initially think that they were fairly abundant. However, our estimation of actual snail density indicated that they were, in

fact, extremely scarce. Only 8 small individuals were found in $22m^2$ of sampled litter and soil, giving a value of 0.36 snails per $m^2$. In Graveland's (1995) study, densities as low as this were only found in forests with high rates of eggshell defects. Since no eggshell defects were observed and the provision of supplementary calcium had no apparent influence on egg production, this would suggest that blue tits are capable of forming normal eggshells even in environments with very low snail numbers and soil calcium levels.

However, our study site was situated in one of the areas worst affected by acid rain in the UK and had exchangeable soil calcium levels lower than that of sites in the Netherlands, where severe eggshell defects occurred. The low soil calcium levels and density of snails may, therefore, be close to a threshold level, below which blue tits would be unable to maintain effective eggshell production. Since proposed reductions in acid deposition in most of Europe are insufficient to halt or decrease anthropogenic soil acidification (de Vries 1994), calcium supply may become a limit on avian reproduction in some areas of the UK in the near future.

To my knowledge, the only other research into acidification and its effects on breeding birds in the UK is work on the Eurasian dipper (*Cinclus cinclus*, Ormerod & Tyler 1987; Ormerod *et al* 1988); an exclusively riverine species which is now known to be scarce on Welsh and Scottish steams with low pH (Ormerod & Tyler 1987). Dippers are dependent on riverine invertebrates for food (Jost 1975; Ormerod 1985; Ormerod & Tyler 1986) and rivers in Northern Britain (Sutcliffe & Carrick 1973)and Wales (Ormerod & Edwards 1987) exhibit marked differences in their invertebrate fauna according to pH. Calcium-rich prey, such as molluscs, crustacea and fish, are frequently very sensitive to acidity (e.g. Okland & Okland 1980; Raddum 1980; Harvey & McArdle 1986; Mills & Schindler 1986) and may be absent or very scarce under acid conditions (Ormerod & Tyler 1986; Turnpenny *et al* 1987). Several breeding parameters of dippers are reported to be depressed along acidic streams by comparison with circumneutral streams (Ormerod *et al* 1991). However, stream pH only accounted for up to 7% of the observed variance in egg shell thickness (Ormerod *et al* 1988).

Perhaps we should also be cautious in assuming that absolute calcium availability is the sole cause of eggshell defects in the Netherlands sites. It is well known that many agricultural and industrial pollutants such as organochlorines, aluminium and heavy metals can impede calcium metabolism during egg laying in birds (Grandjean 1976; Miles *et al* 1993; Nyholm 1981, 1987, 1993; Scheuhammer 1991). The Scottish Highlands have little intensive agriculture or industry compared to the Netherlands and further investigation of the impact of environmental contamination on shell formation in low calcium environments might explain this anomaly between sites (Nyholm 1987).

# CHAPTER 6

## Inter-year and inter-nestbox variation in blue tit breeding parameters

*"I have yet to see a problem, however complicated, which when looked at in the right way, did not become more complicated"*

Paul Anderson (1969, New Scientist)

## 6.1 Introduction

The relationship between food availability and reproductive traits may be moderated by an array of other factors, any of which may subsequently become limiting at high food availability (Martin 1987; Arcese & Smith 1988; Boutin 1990; Schultz 1991; Källander & Karlsson 1993; Wiebe & Bortolotti 1994; Nager, Rüegger & van Noordwijk 1997). Supplementary food might therefore be expected to affect only populations, or individuals within populations, when natural food availability fell below a critical threshold level. Experimental evidence suggests that the provision of supplementary food does generally have the greatest effect when it is provided in a situation where access to natural food is restricted (Nager, Rüeggar & Noordwijk 1997): for example, when natural food levels are low, as a result of an unfavourable year (Dijkstra *et al* 1982) or poor territory (Newton & Marquis 1981), or when population density and, therefore, competition is high (Arcese & Smith 1988).

Similarly, any experimental enhancement of reproductive output induced by supplementary feeding tends to restore levels of breeding parameters towards those that occur naturally when environmental factors are favourable (Boutin 1990). Thus, in Arcese & Smith's (1988) study, supplemental food provided to a high density population of song sparrows restored reproductive output to that of a low density one, whilst in Dijkstra *et al*'s (1982) study of kestrels, supplementary food in a year of low vole abundance restored performance to that of the best pairs in a peak vole year.

Although we do not have long term data on natural food levels for our study population, we do have data on mean laying date, clutch size and egg volume for our study population, over a period of five years. In this chapter, these data are described and used to consider the results of the supplementary feeding studies, presented in *Chapters 3 & 4*, in the wider context of natural variation of unsupplemented birds in the population.

Differing local environmental conditions across a heterogenious habitat can contribute towards variation in reproductive parameters between individuals within a population. The main aims of the analysis presented here were, firstly, to examine whether there was any evidence that particular nestboxes were favoured as nesting sites over others and, secondly, whether individual nestbox sites and territories displayed consistently above or below average breeding performance between years. This might allow any such variation to be excluded from the analyses presented in *Chapters 3 & 4*.

Finally, the presentation of longer term data for our population also permits a more reliable comparison with mean breeding parameter values for other locations and, perhaps, therefore, a better integration of our experimental results with those in the literature. I wish to

make clear, however, that this was never a primary aim of the research and consequently the discussion of, and comparison with, data from other geographic locations is in no way intended to be comprehensive.

## 6.2 Methods and Results

Data for 1994 and 1995 were collected in the course of the supplementary feeding experiments detailed in *Chapters 3 & 4*. Data for 1996 was also collected by myself , however, data for 1992 and 1993 were kindly collected by Caroline Askew. The method of data collection in 1994 and 1995 is outlined in *Chapters 3 & 4*. Data for the other years were collected by visiting all nestboxes at weekly intervals throughout the spring and weighing, measuring and recording all eggs present. Onset of laying dates for 1992 & 96 were estimated from the number of eggs present when a nestbox was visited and the assumption that females lay one egg per day (Perrins 1979).

### 6.2.1 Comparisons between years

#### 6.2.1.1 Onset of laying

I present here a comparison of onset of laying dates between all birds in 1992 and 1996, and control group birds in 1994 and 1995. No data was available for 1993. Original data was normal for all groups except 1994. After square root transformation, however, the data in the 1994 group was normalised and the data in the other groups remained normal. The means presented in Table 6.1a have been back transformed.

**Table 6.1a**: Mean (± SE (n)) onset of laying dates for unsupplemented birds in 1992, 1994, 1995 and 1996.

| Year | 1992 | 1994 | 1995 | 1996 | ANOVA |
|---|---|---|---|---|---|
| Mean onset of laying (in days from April 20th) | 9.50 + 0.91 - 0.88 (43) | 16.70 + 0.93 - 0.90 (30) | 16.96 + 1.20 - 1.16 (20) | 20.03 + 0.40 - 0.40 (61) | $F$ = 39.54, d.f. = 3,150, $P$ <0.00005 |

The results of post-hoc testing are illustrated in Table 6.1.b.

**Table 6.1b**: Results of post-hoc test from Anova in Table 6.1a. '*' denotes a significant difference in a Tukey HSD test.

| | 1992 | 1994 | 1995 | 1996 |
|---|---|---|---|---|
| 1994 | * | | | |
| 1995 | * | | | |
| 1996 | * | * | | |

### 6.2.1.2 Egg Volume

Egg volume was calculated using the equation :

Egg Volume = 0.51× egg length × egg width$^2$ (from Hoyt 1979).

Mean egg volume for the population in a year was calculated using clutch means. Mean egg volumes are presented in Table 6.2 for all birds in 1992, 1993 and 1996 and for control birds in 1994 and 1995.

**Table 6.2**: Mean (± S.E. (n)) egg volume for unsupplemented birds in 1992, 1993, 1994, 1995 and 1996.

| Year | 1992 | 1993 | 1994 | 1995 | 1996 | ANOVA |
|---|---|---|---|---|---|---|
| Mean egg volume ($mm^3$) | 1105 ± 14 (43) | 1084 ± 9 (56) | 1067 ± 16 (29) | 1051 ± 19 (20) | 1093 ± 9 (62) | $F = 2.1357$, d.f. = 4,206, $P = 0.078$ |

From Table 6.2, it can be seen that mean egg size did not differ significantly between years amongst unsupplemented birds, although the Anova approached significance.

### 6.2.1.3 Clutch Size

Clutch size data was transformed by squaring in order to normalise it prior to analysis. Means presented in Table 6.3a. have been back transformed.

**Table 6.3a.**: Mean (± S.E. (n)) clutch sizes of unsupplemented birds in 1992, 1993, 1994, 1995 and 1996.

| Year | 1992 | 1993 | 1994 | 1995 | 1996 | ANOVA |
|---|---|---|---|---|---|---|
| Mean clutch size | 11.06 + 0.35 - 0.30 (43) | 9.86 ± 0.28 - 0.29 (56) | 9.55 ±0.33 -0.36 (29) | 8.65 + 0.52 - 0.60 (20) | 10.76 ± 0.27 - 0.29 (62) | $F = 6.2164$, d.f. = 4,206, $P < 0.0001$ |

The results of post-hoc testing are illustrated in Table 6.3b

**Table 6.3b.**: Results of post-hoc test from Anova in Table 6.3a. '*' denotes a significant difference in a Tukey HSD test.

| | 1992 | 1993 | 1994 | 1995 | 1996 |
|---|---|---|---|---|---|
| 1993 | * | | | | |
| 1994 | * | | | | |
| 1995 | * | | | | |
| 1996 | | | | * | |

### 6.2.2 Investigation of potential nestbox preferences and differences in nestbox quality

#### 6.2.2.1 Nestbox preferences

The pattern of nestbox usage observed over five years is illustrated in Figure 6.1. Each column in this figure represents the number of nestboxes which have been used: never, once, twice, thrice, four times or five times in the course of five years. Also presented in this figure is the expected distribution of nestbox usage if birds showed no preference, and each year selected nestboxes at random from the nestboxes available. The calculations producing this expected distribution are presented in Appendix 2.

It can be seen from Figure 6.1 that the observed pattern of usage is similar to that expected if birds showed no preferences for, or avoidance of, particular nestboxes. There were, however, slightly more unused boxes than expected; slightly less boxes used only once or twice than expected; and slightly more boxes than expected being used three or four times.

#### 6.2.2.2 Nestbox quality

This topic is examined from two different perspectives. Firstly, I investigate whether the degree of variation within nestboxes (i.e. between different years) is significantly less than the variation between nestboxes. Secondly, I investigate whether those nestboxes which have been used on several occasions are actually "better" nestboxes, in terms of the mean egg volume, clutch size and onset of laying date of the birds inhabiting them.

INTER-YEAR CONSISTENCY

One can examine whether the variation between years *within* individual nestboxes is significantly less than the variation *between* nestboxes, simply by carrying out a one-way ANOVA. Data from the five year period, 1992 - 1996, was used. Prior to the analysis, however, data from any nestboxes which had been used only once were removed, since within nestbox variation obviously cannot be calculated on only one value. In addition, records from food supplemented nestboxes in 1994 and 1995 were removed, since this would have affected the intrinsic 'quality' of the nestbox in those years.

**Egg volume and weight**: The mean egg volume or weight per clutch was used in the analysis. For both volume and weight, within nestbox variation was significantly lower than between nestbox variation ($F = 1.534$, d.f. = 76,62, $P < 0.05$; $F = 1.560$, d.f. = 76,60, $P < 0.05$).

**Clutch size**: Clutch size data was square root transformed in order to normalise it, prior to analysis. The subsequent ANOVA was nonsignificant ($F = 0.966$, d.f. = 76,62, n.s.). Clutch size was apparently unaffected by supplementary feeding in 1994, however, and it was, therefore, deemed valid to reinstate the data from food supplemented nests in 1994, in order to increase sample sizes, and re-run the analysis. This ANOVA was, again, nonsignificant ($F = 1.366$, d.f. = 78,85, $P = 0.08$). Clutch size, therefore, did not show any significant evidence of consistency within nestboxes.

**Onset of laying**: Onset of laying data was square root transformed, in order to normalise it, prior to analysis. In an ANOVA, within nestbox variation was not significantly lower than between nestbox variation ($F = 0.832$, d.f. = 69,31, n.s.).

The above analyses examined year-to-year consistency within nestboxes in terms of the actual values of reproductive parameters. The mean value of these parameters for the whole population, however, can vary from year to year (most probably as a result of environmental factors). Therefore, it may be that nestboxes exhibit year-to-year consistency in terms of their relationship to / deviation from, the population mean each year; rather than the absolute level of a reproductive parameter. For example, although clutch size may vary from year to year at a particular nestbox, it may be consistently larger or smaller than the population mean. In order to investigate this, all data was converted to residuals by subtracting the mean value for the population in that year. In 1994 and 1995, when only data from control pairs was used, the population mean used was the mean for the control group only. Again, only data from nestboxes occupied more than once was included. ANOVAs on residual clutch size and onset of laying date were again non significant ($F = 1.074$, d.f. = 76,62, n.s.; $F = 1.455$, d.f. = 69,31, NS, respectively). An ANOVA on residual egg volume, however, approached significance ($F = 1.494$, d.f. = 76,62, $P = 0.052$). The use of residuals, however, did not result in any improvement in statistical resolution: in fact, an ANOVA on residuals only approached significance, whilst that using absolute data was significant. It would, therefore, be parsimonious to conclude that the intra-nestbox continuity observed is, in reality, a function of actual egg volume rather than relative egg volume.

## IS FREQUENCY OF USAGE RELATED TO NESTBOX 'QUALITY' ?

If nestboxes differ in 'quality' such that birds nesting in high quality nestboxes have greater reproductive success, and birds are able to detect high quality nestboxes, then if there are more nestboxes than pairs, it follows that a high quality nestbox would tend to be occupied more frequently than a low quality nestbox. This section examines whether mean values for egg volume, clutch size and onset of laying differ significantly between nestboxes according to their frequency of occupation.

For nestboxes occupied more than once, the mean value for all occupied years is used in the analysis. As before, clutch size data was square transformed and onset of laying data square root transformed, in order to improve normality, prior to analysis. Nestboxes occupied either three or four times were placed in one category and those occupied either once or twice in another. The results of t-tests on data in these categories are presented in Table 6.4. Means for clutch size and laying date have been back transformed.

**Table 6.4**: Means (± SE (n)) for reproductive parameters by grouped frequency of occupation.

| Reproductive parameter | Nest boxes occupied once or twice | Nestboxes occupied three or four times | t-test |
|---|---|---|---|
| Egg volume | 1085 ± 7 (120) | 1094 ± 14 (27) | t = 0.60, d.f. = 145, n.s. |
| Egg weight | 1.129 ± 0.007 (120) | 1.151 ± 0.017 (27) | t = 1.24, d.f. = 145, n.s. |
| Clutch size | 9.94 ± 0.18 (120) | 10.74 +0.31 - 0.32 (27) | t = 2.02, d.f. = 145, $P = 0.045$ |
| Onset of laying date | 16.68 + 0.67 (94) | 14.04 + 1.02 - 0.99 (27) | t = 2.00, d.f. = 119, $P = 0.048$ |

The analyses presented in Table 6.4 detected significant differences in both mean clutch size and onset of laying date between those nestboxes occupied three of four times and those occupied only once or twice; being larger, and earlier, respectively, in the more frequently occupied category.

From previous analyses (*Chapters 3 and4*) however, it is known that clutch size and laying date are related; mean clutch size declining through the season. It is possible, therefore, that the observed difference in mean clutch size is simply a result of the earlier laying date. In order to test this an analysis of covariance was carried out on clutch size data, with frequency of occupation as the factor ('occupied once' versus 'occupied three or four times') and laying

date as the covariate. In this analysis, the relationship between clutch size and laying date was highly significant, and when this was taken into account, there was no significant effect of frequency of occupation on mean clutch size ($F_{\text{reg.}}$ = 13.11, d.f. = 1,75, $P$ <0.001; $F_{\text{factor}}$ = 1.410, d.f. = 1,75, NS.; $F_{\text{model}}$ = 8.89, d.f. = 2,75, $P$ < 0.0005).

Thus, mean onset of laying date is significantly earlier amongst nestboxes used frequently in comparison to those used infrequently, and mean clutch size differs significantly between these categories as a function of laying date.

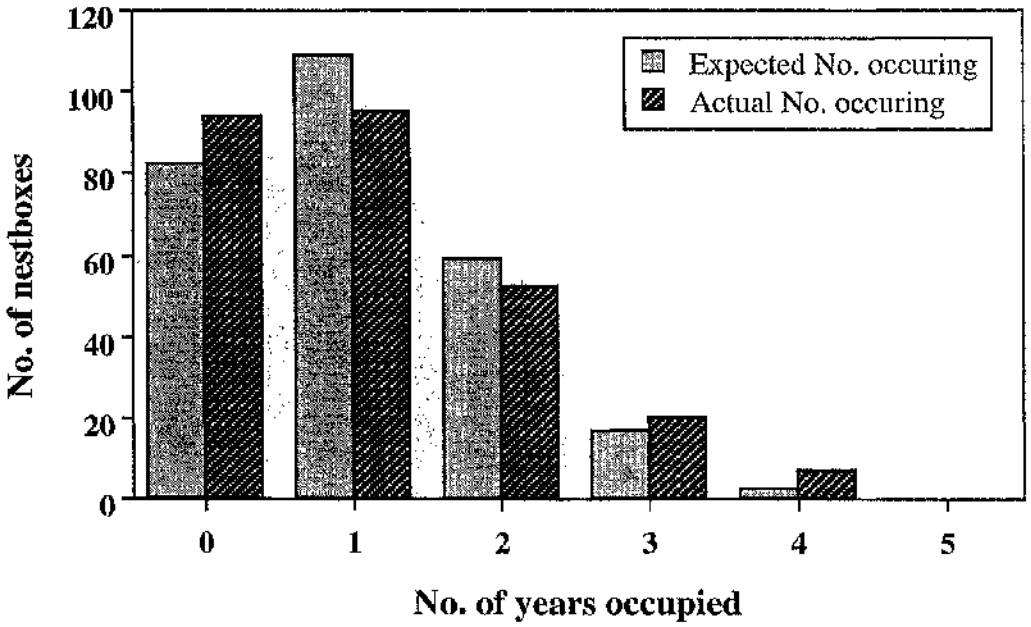



**Figure 6.1**: Observed and expected frequency of nestbox usage over five years at study site

## 6.3 Discussion

### 6.3.1 Comparisons between years

Mean laying date varied significantly between years, by a maximum of about 10.5 days. This natural variation was much greater than that produced by supplementary feeding experiments in the same population (*Chapters 3 & 4*). In 1994 and 1995, supplementary feeding advanced mean onset of laying date by approximately 2 - 3 days; mean laying date of control groups in these years, however, was approximately 7 days later than in 1992. Thus, although onset of laying date is influenced by photoperiod (Suomailainen 1937; Farner & Wingfield 1980) and food availability, above certain critical limits the decision to commence breeding is moderated by other factors (Svensson & Nilsson 1995; Boutin 1990; Martin 1987).

Mean clutch size of unsupplemented birds also showed significant variation between years; ranging from 8.7 in 1995 to 11.1 in 1992. Interestingly, supplementary feeding resulted in a significant increase in mean clutch size in 1995; the year of smallest mean clutch size amongst controls. It is tempting to postulate that perhaps the most scope for an experimental increase existed in this year. There is evidence from the literature, that the provision of supplementary food generally has the greatest effect on clutch size when it is provided in a situation where access to natural food is restricted (Nager, Rüeggar & Noordwijk, 1997). In the absence of data on natural food levels, clutch size of controls has often been used as a indicator of natural food availability. However, supplementary feeding did not result in an increase in clutch size in 1994, yet the clutch size of control birds in that year was significantly smaller than clutch sizes of unsupplemented birds in 1992. The lack of an experimental increase in clutch size would not, therefore, appear to be because clutch size was already close to some maximum limit for the population; perhaps determined by genetics or maximum brood rearing capacity. Neither is it likely to be due to the quality of the supplementary food used (*Chapter 4*). Rather, factors other than food availability must have been acting to maintain mean clutch size at a level below the population maximum. Mean clutch size of controls, therefore, may not always be a good indicator of relative food abundance between years (Nager *et al,* 1997)

Mean clutch size in the experimental group receiving high quality protein in 1995, increased to a level similar to the largest mean clutch sizes observed for unsupplemented birds in any of the five years. This supports the suggestion that any experimental enhancement of reproductive output induced by supplementary feeding, tends to restore levels of breeding parameters towards those that occur naturally when environmental factors are favourable (Boutin 1990; Arcese & Smith 1988; Dijkstra *et al* 1982)

Although mean egg volume varied significantly from year to year, it did so much more conservatively than other reproductive traits examined. Consequently, no statistically significant differences in mean egg volume were detected between years. Supplementary feeding, with an egg-based diet, resulted in a significant increase in mean egg volume in 1994. The mean volume of eggs in this experimental group was greater than that for control groups in any of the five years for which data was available. Although greater than the mean egg volume of controls in 1992 (year of largest mean egg volume amongst control birds) the difference was not significant. Therefore, the provision of high quality supplementary food in 1994, may have increased/restored mean egg size to maximum level that would occur in the population if environmental factors were favourable (analogous to the situation discussed above for clutch size); rather than producing supernaturally large eggs. Supplementary feeding in 1995 did not result in any increase in mean egg volume. Mean egg volume of birds in the control group in 1995, however, was lower than in 1994 and, in fact, was the lowest of the five years for which data was available. It would appear, therefore, that there was sufficient physiological scope for mean egg volume to increase in 1995; supporting the hypothesis discussed in *Chapter 4*, that either the supplementary diets did not provide sufficient of a limiting nutrient, or egg volume was determined, at this lower level, by other factors.

### 6.3.2 Comparisons with other populations

CLUTCH SIZE

Across their geographic range, blue tits have commonly been found to exhibit clutch sizes of between 2 to 18 eggs (Cramp & Perrins 1993). In the south of the range, average clutch size can get extremely low; around 3.5 eggs per clutch in Tenerife, Canary Islands (Isenmann 1987b). Over much of continental Europe, however, average size of first clutches is mostly 10 - 12 eggs (Berndt *et al 1983*). Average clutch size for our population, over five years, was about 10 eggs. This is similar to the reported average clutch size of 10 - 11 eggs in the deciduous forests of Wytham wood, near Oxford (Perrins 1979), and the Revinge area of southern Sweden (Nilsson & Svensson 1993a,b; Nillson 1994). These areas are mentioned, in particular, because they are other sites of considerable research into the influence of food supply on reproduction in tits. Data for other Scottish blue tit populations is scarce. However, means of around 10 eggs are reported for populations breeding in deciduous woodland around Argyle, and near Perth (Petty 1989; Greenwood & Hubbard 1979). In addition, a mean clutch size, over five years, of 9.1 was reported for blue tits breeding on the west of Scotland island of Rhum (Love 1981).

However, it has been suggested that clutch size is probably lower on northern islands than on mainland at the same latitudes (Cramp & Perrins 1993).

Factors affecting clutch size in blue tits were considered, in some detail, in *Chapter 4*. That discussion, however, concentrated primarily on inter-individual differences within a population. I here consider, briefly, the main factors resulting in consistent differences in mean clutch size between geographically distant populations; habitat and latitude.

Considering habitat first, the principal distinction occurs between deciduous and evergreen woodland: mean clutch size generally being considerably smaller in evergreen (van Balen 1973; Zandt *et al* 1990; Blondel *et al* 1993). This difference occurs largely as a result of differences in food levels. In oak woodland, for example, caterpillar abundance and size increases rapidly in a brief boom after the buds of the leaves, upon which they feed, burst in the spring. Such rapid development occurs because deciduous trees combat insect depredation of their leaves by dramatically reducing leaf palatability (by reducing water and protein content and increasing the concentration of toxic secondary compounds, such as tannins) shortly after leaves are formed (Feeny 1970). Tit breeding, consequently, is timed to take advantage of this burst in prey availability. A similar 'race between the species' occurs with other deciduous trees and their associated invertebrate foliavores; however, it occurs to a much lesser extent in evergreen trees, since there is not such a brief time window of new leaf development (Speight & Wainhouse 1989). As a result, caterpillars occur there at a lower density, but for a longer period of time. Consequently, clutch size and breeding success are generally lower there, but birds often have more opportunity to raise additional broods (Van Balen 1973). Perrins (1979), also reports that mean clutch sizes of tits are significantly lower in gardens than deciduous woods as a result of the much poorer food supply there; and there is evidence that, for blue tits, clutches are smaller in mixed woodland than in woods which are predominantly oak (Perrins 1979; Isenmann 1987b).

I turn now to our second main factor, latitude. It has long been recognised that clutch size increases with latitude (Lack 1947, 1954; Hussell 1972; Berdt *et al* 1983; Koenig 1984, 1986; Isenmann 1987b; Young 1994). Latitude itself, however, is not an environmental variable and some environmental variable(s), correlated with latitude must, therefore, be responsible. Among the principal environmental factors proposed to explain the relationship, are daylength (Lack 1947, 1954; Hussell 1972) and food abundance (Ashmole 1961; Ricklefs 1980; Martin 1987).

Given constant food abundance, for animals providing parental care, increased time and energy spent foraging for young either produces improved growth and/or condition of the young, leading to greater survival probabilities (Martin 1987, 1992; Linden & Moller 1989), or permits the raising of more young. However, increased time spent foraging for young may

conflict with other activities such as nest defence, rest or self maintenance (Cody 1966; Ricklefs 1970; Yom-Tov 1974; Hogstedt 1980; Martin 1992). Lack (1947, 1954) proposed that increased day length during the breeding season, in more northerly latitudes, allowed parents to collect more food and thus raise more young; whilst also allowing parents more time for the other activities above.

On the other hand, an increase in overall food abundance can also lead to an increase in optimal clutch size (Martin 1987). Food abundance is known to vary with latitude; largely as a result of variation in vegetation type. The main effects relate to the differences between deciduous and evergreen forest discussed above: in more northern and equatorial latitudes, evergreens predominate; whilst in more central latitudes deciduous forests are more common.

A study by Belda (1997), using data from over 60 blue tit populations, examined these alternative hypotheses and concluded that the variation in clutch size with latitude was a result of the interaction of latitudinal variation in vegetation and daylength.

The relationship between clutch size and latitude, although significant over the whole range of the blue tit (28° N to 65° N, Cramp & Perrins 1993), generally shows no particularly clear trend between 45° - 60° N (Belda 1997). However, a slight increase from north to south, of 0.315 egg per degree latitude, was found at 50 - 55° N in West Germany (Berndt *et al* 1983). If a similar latitudinal trend existed in Britain, one would expect average clutch size at our study site (56° 9' N) to be approximately 1.4 eggs larger than in Wytham wood, Oxfordshire (51° 46' N). Although relevant data is limited, this does not appear to be the case.

## LAYING DATE

I shall not discuss at any length here, differences in mean laying date between populations in geographically distant locations. Average laying dates of first clutches range from early April to mid-May (Cramp & Perrins 1993). Latitude (Berndt *et al* 1983), photoperiod (Suomailainen 1937; Farner & Wingfield 1980), altitude (Zang 1982a; Berndt *et al* 1983), environmental temperature and weather (Kluyver 1952; Lack 1958; Källander 1976; Love 1981; Schmidt 1984), breeding density (Arcese & Smith 1988), habitat (Lack 1958; Dhont *et al* 1984), female age (Källander 1976) and food supply are all environmental factors that have been shown to influence timing of breeding in passerines. Comparisons of actual mean calendar date between populations are often not particularly useful without information on how these laying dates relate to other local environmental factors, such as timing of prey availability.

Over much of central and northern Europe, laying begins mostly in the last week of April and first few days of May (Cramp & Perrins 1993). A retardation of 2.5 days per degree

of latitude, at 50° - 55° N, is reported by Berndt *et al* (1983). Applied to Britain, this would predict a difference of 11 days in average laying date between my study site at Rowardennan and Wytham wood, Oxfordshire. Average laying date, over 29 years, for southern England is reported as 25th April (Dunn 1976), and, over 5 years, at Rowardennan was around 6th May; a difference of 11 days, as predicted.

### 5.3.3 Variation in nestbox usage and quality

From Figure 6.1, it can be seen that the observed pattern of nestbox usage, over five years, was not dramatically different to that expected if breeding birds chose nestboxes at random, from those available. However, there were slightly more unused boxes than expected; slightly less boxes used only once or twice than expected; and slightly more boxes than expected being used three or four times. Although fairly small, this is the pattern of deviation from expected distribution one would expect if a small degree of nestbox preference were occurring. However, it does not necessarily indicate that nestboxes differ in intrinsic quality or 'desirability'. Birds surviving to breed in more than one year may simply exhibit a degree of site faithfulness, tending to return to the same nestbox year after year (C.M. Perrins, personal communication).

Mean laying date was significantly earlier amongst nestboxes used frequently, in comparison to those used infrequently. Mean clutch size also differed significantly between these categories, as a function of laying date; being larger amongst nestboxes used more frequently. This result might suggest that different nestbox sites vary in quality (either the quality of the nestbox itself or of the habitat/territory within which it is situated) and that higher quality sites are used more often. There are two mechanisms by which such a relationship could come about. Firstly, differences in nestbox site quality could have a direct influence on the mean date of laying; for example, a higher quality area may receive more sunlight and be less exposed to wind, with the result that bud burst and caterpillar development occurs earlier, permitting earlier formation of eggs. Alternatively, differences in nestbox site quality might influence laying date indirectly. High quality females may obtain high quality nestboxes/territories, but may also be able to lay earlier as a result of, for example, better foraging abilities or better body reserves.

The above hypotheses cannot be distinguished, because adult females were not individually marked and, therefore, we have no record of individual female quality. However, regardless of which hypothesis were correct, one would still expect that food supplementation would have a greater effect at lower quality nestboxes.

There is an alternative explanation for this result, however, which is unrelated to differences in nestbox quality. As mentioned earlier, females which survive to breed in more than one year may exhibit a degree of site faithfulness; tending to return to the same nestbox to breed each year. Further, there is evidence that individual females tend to lay earlier as they get older (Perrins 1979). Thus, the mean lay date in boxes occupied more frequently may be earlier simply as a result of the earlier laying of older birds.

In addition to examining the above relationship between frequency of nestbox occupation and mean values for reproductive parameters, I also considered the related topic of inter-year consistency of reproductive parameters within nestboxes. In these analyses, egg volume displayed significantly less variation between years within individual nestboxes, than it did between individual nestboxes. This implies some consistency in egg volume within nestboxes between years. Laying date and clutch size, however, showed no evidence of consistency within nestboxes.

Again, there are two main hypotheses to explain the observed consistency in egg volume. Different nestbox sites may vary in quality, and this in turn may influence egg volume; either directly or indirectly, as discussed above. Alternatively, egg volume may be fairly consistent within females, from year to year, and females may show breeding site faithfulness. The fact that mean egg volume did not show any significant difference between nestboxes occupied only once or twice and those occupied three or four times, indicates that egg volume is unrelated to nestbox quality. If good quality females also tend to obtain good quality nestbox sites, then egg volume would not appear to be related to female quality either. This leaves us, again, with the site faithfulness hypothesis. There is evidence that repeatability values between different clutches of the same female are generally high (van Noordwijk *et al* 1981; Grant 1982; Wiggins 1990). If females surviving to breed in more than one year, show some tendency to return to the same nestbox to breed, this would explain the observed result.

When analysing results from food supplementation experiments, the inclusion of nestbox quality or female age, as an additional factor, could perhaps give greater statistical power to detect induced effects. For the experimental studies presented in *Chapters 3 & 4*, however, sample sizes of data from previous years, relevant to the nestboxes occupied during the experiment, were too small to allow an attempt at reliable classification of all experimental nestboxes in terms of quality. Adequate information on female age was similarly unavailable. Consequently, I was unable to utilise the technique.

# CHAPTER 7

## General Discussion

*"There is a theory which states that if ever anyone discovers exactly what the universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarrely inexplicable.*

*There is another theory which states that this has already happened."*

Douglas Adams (*The Hitchhiker's Guide to the Galaxy*)

## General Discussion

The aim of this study was to investigate the role of exogenous nutrient availability in determining timing of breeding and level of investment in egg production in a population of blue tits. Previous studies, examining the relationship between food supply and breeding, have tended to concentrate on the energetics of reproduction. In this study, I have attempted to consider all components of nutrition, but have devoted particular investigative endeavour to the topic of protein availability and quality. Other potentially limiting dietary nutrients for breeding birds are essential fatty acids, vitamins and minerals. Vitamins, however, are only required in trace quantities and are very unlikely to be limiting in a non-artificial diet. Essential fatty acids although required in relatively small quantities, can limit egg production in poultry. I have no data on essential fatty acid requirements of blue tits or potential levels in the natural diet and, therefore, cannot exclude essential fatty acids as a potentially limiting nutrient. However, as discussed in *Chapter* 4, there was no evidence from supplementary feeding experiments for essential fatty acids constraining egg production, as the only essential fatty acids known to be necessary for optimum egg production in poultry were present in abundance in all the diet supplements used. Of a laying bird's mineral requirements, calcium is the most likely to become limiting first. The experimental provisioning of supplementary calcium, however, provided no evidence that egg production at our study site was constrained by calcium availability (*Chapter 5*).

The influence of food supply, especially energy and protein supply, prior to and during the egg laying period, on timing of breeding and level of investment in egg production has been discussed in detail in *Chapters 3 & 4*. To avoid needless repetition, I shall not, therefore, reconsider these points at any length again here. In this 'General Discussion' I shall, instead, take the opportunity to consider the nutritional requirements of a laying blue tit; the energy and nutrient sources which may be available to a tit at this time; and the physiological, behavioural and environmental factors and interactions which may explain the findings of the previous chapters.

In *Chapter 2* I presented an estimate of daily essential amino acid (EAA) requirements for a laying blue tit, and the minimum mass of lepidopteran larvae which would be required to satisfy this requirement. I now present below an estimate of daily energy expenditure for a laying blue tit in order to allow an examination of whether EAA or energy would be expected to become limiting first, on such an invertebrate diet.

## Estimated daily energy requirement

ENERGY REQUIREMENT FOR MAINTENANCE

Presented below are three estimates of daily energy expenditure:

a) Average daily energy expenditure for maintenance has been estimated to be 2.6 - 2.7 × BMR (Drent & Daan 1980; Aschoff & Pohl 1970a)

BMR was estimated in *Chapter 2*, from the equations of Aschoff & Pohl (1970b), to be 5.72Kcal/day (= 23.93 kJ/day).

Thus, DEE = **62.2 - 64.6 kJ/day**

b) Robbins (1993) gives an equation, derived from measures using doubly labelled water, relating body mass to daily energy expenditure (DEE) for free-ranging passerines:

DEE (Kcal/day) = 338.9 × mass(Kg) $^{0.72}$

The average mass of a female blue tit during laying (including reproductive tissue, but excluding any fully or partially formed eggs) is about 12.0g (Perrins 1979).

Thus, DEE = $338.9 \times (0.012)^{0.72}$

= 14.03Kcal/day = **58.70kJ/day**

These equations, however, are general for all passerines and take no account of varying environmental temperature.

c) Gibb (1957) gives the daily energy expenditure of captive blue tits in winter (estimated from food consumption) at three different ambient temperatures.

39.7 kJ/day (av. air temp. = 11° C); 44.3 kJ/day (av. air temp. = 7° C);

51.4 kJ/day (av. air temp. = 3° C)

7° C is probably close to average air temperature around the time of laying (Mertens 1987), although it may be slightly higher. The values quoted by Gibb were for birds weighing 10 - 11g. Assuming an average mass of ~10.5g, and a mass relationship of $M^{0.72}$ (from above), this equals $(^{12}/_{10.5}) \times 44.3$ kJ/day = **48.77kJ/day** for a 12g bird.

Although this estimate is specific for blue tits, the activity levels of free-ranging birds (particularly those associated with foraging) would probably be somewhat higher than those in captivity.

The actual value of $DEE_{(maintenance)}$ for a free-ranging, laying blue tit probably lies somewhere around the estimates above. Considering the relatively high costs of flight, the estimates derived from equations for free-ranging birds (a, b) are probably closer to the actual value of $DEE_{(maintenance)}$ for a laying blue tit than values obtained from captive birds (c), despite the generalisation for temperature. A mean value equal to (58.70 + (62.2 + 64.6)/2)/2 = **61.0kJ/day** will thus be used as a rough estimate in the following calculations.

ENERGY REQUIREMENT FOR EGG PRODUCTION

Mean fresh egg weight for blue tits in our study population was 1.138g and the mean fresh weight of constituents: 0.083g shell, 0.262g yolk and 0.793g albumen (*Chapter 2*). No data on energy content or lipid content was available for these constituents, but protein contents had been determined and these agreed fairly closely with the protein contents given by Yoo (1993) for great tit egg constituents. Therefore, the energy content of blue tit yolk and albumen was calculated using the respective energy densities given by Yoo (1993).

Thus, energy content of albumen $= 2.519\text{kJ/g} \times 0.793\text{g}$

$= 1.996\text{kJ}$

& energy content of yolk $= 15.435\text{/g} \times 0.262\text{g}$

$= 4.046\text{kJ}$

Therefore, energy content of a single whole egg = 6.042kJ.

The efficiency of energy deposition by a small passerine into eggs is reported to be approx. 75% of metabolizable energy intake (El-Wailly 1966). The total energy required to form one egg would, therefore, be (100/75) × 6.042kJ = **8.056kJ**.

As discussed in *Chapter 2*, each egg takes 3-4 days to form, mainly due to the time required for ova growth. King (1973) showed that in clutch forming species in which one egg is laid each day, the peak daily metabolic output for egg production during the laying period is equivalent to the total investment in a single egg.

Thus, if a female blue tit laid one egg per day, the estimated total daily energy expenditure would equal:

61.0kJ/day + 8.1kJ/day

**= 69.1 kJ/day**

## Energy and EAA requirements in relation to dietary arthropods

From *Chapter 2*, the mass of small caterpillars required to satisfy daily SAA requirements would equal = 798mg

The average energy content of terrestrial arthropods is 22-24 J/mg dry weight (Gibb 1957; Golley 1961; Kale 1965; Bryant 1973; Kendeigh *et al* 1977; Norberg 1978; Bell 1990; Robel *et al* 1995) with relatively little variation between orders. However, Karasov (1990) reports that only around 19.3 J/mg of this is metabolizable energy. 798mg of 26th May caterpillars would, therefore, be expected to provide 15.4 kJ. This would supply only 64% of estimated BMR or 22% of estimated daily energy requirements during laying. For a blue tit to obtain sufficient energy it would therefore need to consume considerably more invertebrates than required to satisfy the minimum estimated requirements for all essential amino acids.

Murphy (1994), carried out similar calculations for a model 25g insectivorous passerine, and also concluded that it was unlikely that essential amino acids would limit egg production because the food intake required to meet energy requirements would exceed the minimal requirements for all essential amino acids. However, the supplementary feeding trial reported in *Chapter 3* demonstrated that, although the timing of breeding could be influenced by energy supply, egg production was proximately constrained by nutrient availability. That specific amino acid availability could proximately constrain egg production was confirmed by a further experiment, detailed in *Chapter 4*. It is, perhaps, not surprising that a bird, such as the zebra finch (*Taeniopygia guttata*), feeding on a diet known to be low in total protein and where that protein is of low quality, should be constrained by nutrient availability (Houston *et al* 1995a,c; Selman & Houston 1996). It is, however, remarkable that birds feeding on an animal diet, of high protein quantity and quality, such as lesser-black-backed gulls (*Larus fuscus*, Bolton *et al* 1992) or tits should demonstrate such a dietary constraint.

There may, however, be a number of assumptions made in the rather simplistic balance calculations which infer that a comparatively high intake of essential amino acids is taking place, which are false.

Firstly, we are assuming that the birds' diet is entirely insectivorous at the time of egg formation. In section 2.4, I discussed the fact that arthropod abundance, particularly phytophagous arthropods, is closely related to bud burst. Different tree species come into leaf at different times, and oak is comparatively late. Therefore, early in a mixed wood, tits can feed on arthropods from other tree species and switch to oak later in the season when arthropod abundance has increased. In an oak-dominated wood, however, blue tits' ability to switch trees early in the season is severely restricted and they may thus be expected to experience an

extremely low availability of invertebrates. The scarcity of invertebrates at the start of the laying season was confirmed in *Chapter 2*. Consequently, tits may also have to feed on other, non-invertebrate, food items early in the season.

Betts (1955) investigated Parid diets, by gizzard analysis, throughout the year in an oakwood in south west England. In March and April, the gizzards of all blue tits examined ($n$=26) contained greater than 40% (by volume) oak bud tissue; and over two thirds of these birds' gizzards contained volumes of bud tissue greater than 75%. By contrast, tits breeding in a mixed wood, were found to contain an average of only 9% plant tissue in their gizzards at this time of year (Woodburn 1997). One cannot rule out that this plant tissue is taken unintentionally whilst searching for eggs, larvae or pupae of arthropods contained within the bud. However, Betts (1955) draws attention to the fact that Cynipid larvae (which were the main inhabitants of the oak buds during her study) were found in less than 25% of the birds containing oak tissue; indicating that the plant tissue may form an intentional part of the diet. In addition, blue tits are extremely dextrous in their manipulations of prey and other objects in the bill (Perrins 1979), and it is difficult to believe that they could not separate an insect grub more efficiently from the surrounding leaf tissue before swallowing it.

Why then should blue tits feed on bud tissue at this particular time in the year? Betts (1955) did not record oak tissue as a significant portion of gut contents at any other season of the year.

It may be that leaf-buds, at this time, contain some essential nutrient(s), such as trace levels of vitamins. However, the volume consumed suggests the provision of macro-nutrients. As discussed in *Chapter 2*, plant tissue can only partially be digested by vertebrates which do not have a specially adapted gut with specialised fermentation regions for symbiotic micro-organisms. The cells of bud tissue and young leaves, however, have much thinner, more delicate cell walls than older leaves or structural tissue and are much richer in protoplasm (Knight & Step 1905; Esau 1977). Therefore, tits may be able to mechanically rupture many of the cell walls in this tissue and gain access to the relatively rich protoplasmic contents. Although the protein content of early growth in plant tissue can be higher than at maturity, it is still usually considerably lower than in animal tissue. Further, plant-derived protein is also of considerably lower quality - containing comparatively little sulphur amino acids or lysine (Robbins 1993; *Chapter 2*). Oak buds would therefore be a food source of low protein quality and quantity. In contrast, the relatively concentrated protoplasm in bud tissue may be a comparatively rich energy source. Berggren (1985) reports that the relative volume of lipid bodies and starch granules is higher in dormant buds than in growing tissue. Also, cytoplasmic sugar concentrations may be considerably elevated in order to reduce the likelihood of cell contents freezing. In addition, young tree buds are often coated with a balsam (i.e. a solution of

resin in an ethereal oil) to protect them from cold and wet during winter and early spring (Knight & Step 1905). Such plant resins could add significantly to the overall energy content of leaf-buds, if blue tits were able to digest them. Although there is no relevant information available regarding such digestive capabilities in blue tits, there is evidence that grouse digest large quantities of fairly resinous forage during the winter (Bryant & Kuropat 1980). Blue tits may, therefore, deliberately ingest leaf-bud material because of a high and *accessible* (sugar, starch and lipid are comparatively easy to digest; although digestibility of resin is unknown) energy content. The scarcity of invertebrates may make this necessary, in order for the blue tit to satisfy daily energy requirements.

Further evidence to support such a hypothesis comes from records of blue tits drinking the sap of birch and other trees (Mylne 1959; Bardin 1987; Hund 1974) and the nectar from flowers of several trees and other plant species (Ford 1985; Kay 1985; Richards 1986; Johnson 1985; Visick 1977; Vogel *et al* 1984) at this time of year. It is difficult to say exactly how great a contribution this might make to the diet. Observational studies of foraging location may not often be able to distinguish whether a bird foraging at buds/twigs is taking invertebrates, plant tissue or sap/nectar. Further, studies examining stomach contents (e.g. Betts 1955; Woodburn 1997) make conclusions on the basis of undigested material found there, e.g. leaf material or components of exoskeleton from arthropods. Ingested sugar solution, would be completely undetected. Consequently, it is quite possible that an important component of the diet at egg laying time has been largely overlooked.

Some indication of the contribution these activities may make to the daily energy intake is given by Kay's (1985) observations of blue tits feeding at the nectar of Willow catkins. Tits repeatedly drank nectar 15-45 times on most catkins; visiting an average of 9-10 catkins per minute; and were seen feeding for up to 14 minutes at a time. Further, the longest visits occurred during 06.45 - 08.30 hrs and 16.00 - 18.00 hrs. This corresponds to the periods when a bird's dietary energy requirements may be greatest; replenishing energy stores depleted during the night, and building up energy reserves in preparation for the coming night. Some circumstantial support for blue tits exploiting such an energy source at our study site comes from observations in *Chapter 2.* Although by late May, tits were observed to forage almost exclusively in oak; during mid-April - early May, they spent approximately 23% of observed foraging time at birch. Subsequent sampling of a large amount of birch twigs revealed an extremely low abundance of arthropods. However, as mentioned before, there are records of blue tits feeding avidly at the sap of birch trees. The spring sap of the birch is so rich in sugar that it can be fermented to form an alcoholic liquor, or else evaporated to produce a solid sugar (Edlin 1973; Milner 1992). To give an idea of its plenitude, production in Russia, in 1986, amounted to 42,700 tons and silver birch wine is also made commercially in the Highlands of

Scotland (Milner 1992). It is quite possible, therefore, that tits observed foraging at birch were, in fact, feeding on the very high energy sap.

Such sap and nectar, however, contains extremely low levels of amino acids in relation to its sugar/energy content (Paul & Southgate 1979; Paul *et al* 1979; Karasov 1990). Oak leaf-bud tissue, as discussed earlier, may also yield comparatively little protein in relation to its available energy, and what protein tits can gain is likely to be of low quality in terms of sulphur-containing amino acids and lysine. Tits may, therefore, rely on these energy-rich plant-derived foods to allow them to forage selectively for scarce invertebrates as a source of limiting EAA.

Pressure to feed on these high energy, but low protein quality foods may also partly be caused by the high calcium intake required at laying time to form eggshells. Although *Chapter 5* did not provide any evidence that calcium supply constrained egg production, it demonstrated that soil calcium levels were very low around our study site and that snails were, correspondingly, very scarce. There is evidence that tits may have to spend a considerable period of time each day foraging for calcium-rich items. Graveland & Berends (1997) report that captive female great tits spent 20% of their time handling and eating snail shells, and egg-laying female goldcrests spent at least one hour per day collecting small snail shells. Some selective foraging on high energy food items may be required to fuel this search for calcium. We might conclude, therefore, that the assumption made in the balance calculations that tits are feeding on a diet exclusively composed of insect tissue at the time of egg laying may be false.

A second basic assumption behind the simplistic balance calculations, is that the estimates used for efficiency of digestion, efficiency of nutrient absorption, and efficiency with which absorbed nutrients are converted into egg material are relatively accurate. In actual fact, we know almost nothing about any of these processes in the tit, or for any wild bird. Research by Gibb (1957) demonstrated an apparent metabolizable energy co-efficient (MEC = % of total energy in ingested food obtained for use by the animal) of 0.84 and 0.86 for mealworms consumed by blue tits and coal tits (*Parus ater*), respectively. However, MEC for mixed arthropods fed to coal tits was only 0.67. Such differences may be primarily due to differing proportions of relatively indigestible cuticle; a component which may comprise 1-50% of dry matter depending on arthropod species and stage of development (Karasov 1990). For example, in carnivores it has been demonstrated that the digestible energy coefficients for adult insects (~77%) are considerably lower than for invertebrate larvae (~91%) (Robbins 1993). Thus, caterpillars may provide more energy per unit mass than has been assumed in calculations. Similarly, there is evidence that the utilisation efficiency of nectar (and perhaps, therefore, sap) is practically 100% in avian nectarivores (Karasov 1990), although data are lacking for birds in which nectar makes up a smaller proportion of the diet. Research on wild species of birds,

however, has tended to focus almost exclusively on the efficiency of energy extraction and utilisation (Robbins 1993; Karasov 1990) and very little information is available regarding amino acids. Figures in the literature are often derived from poultry research (Leville *et al* 1960; Scott *et al* 1982), and the little research that has examined protein utilisation in wild species has tended to be done on either wildfowl or captive and largely granivorous passerines (El-Wailly 1966; Murphy 1994). We, therefore, have virtually no reliable data on whether they are applicable to small insectivorous passerines.

Interestingly in this context, there is evidence that gut morphology of blue tits changes between winter and summer (Perrins 1979). It is well known in other species of birds and mammals that changes in gut morphology associated with dietary change can have important implications for digestive efficiency (Moss 1974; Drobney 1984; McLelland 1991; Lee & Houston 1994). The main change is in the weight of the gut wall which becomes more muscular in winter - possibly in order to be able to grind up a harder, more seed-dominated diet at that time of year (Perrins 1979). If in spring, gut morphology has changed, or is in the process of changing, towards an anatomy more suited for handling an insectivorous diet, blue tits may be less efficient at digesting plant material than they are in the winter. Further, the enzymes required for efficient digestion of the different food types will be different and the tits may experience problems if enzymes have begun changing towards those more suited to animal tissue, which will form most of the diet later in the breeding season. Again, this may prevent them from utilising efficiently foods which they had been exploiting during the winter. More information and research is required regarding the values of digestion, assimilation and conversion parameters, especially for essential amino acids. Variation in the efficiency of any of these parameters may alter our calculations substantially and, thus, the predicted probability of EAA limitation.

A third major assumption is that, if physiological digestive efficiency figures used in these calculations are valid for a normal blue tit, they will not change substantially at the time of laying. Egg laying is a time of substantial physiological stress for the female, and many changes may occur in her body and in the efficiency of her metabolic processes. There are also two major changes taking place in a blue tit's diet at laying time which may cause digestive physiology to change.

Firstly, many birds appear to ingest large quantities of oak leaf-bud tissue at this time and, as discussed above, may therefore experience difficulty in producing appropriate enzymes to deal with both plant and invertebrate protein. There are, however, additional considerations, relating to such diet mixing. The ingestion of a greatly increased volume of bulky, less digestible material may result in the faster passage of all food through the gut. Reduced

residency time and increased bulk of indigestible matter may, consequently, result in a decrease in the efficiency with which nutrients are extracted from ingested invertebrates.

The soluble phenolics known as tannins, which are present in the leaves of oak, may also reduce protein availability. Tannins act by binding to ingested proteins and precipitating them - rendering them inaccessible to the animal. Although tannin concentration only reaches high levels after leaves are fully formed (Feeny 1970), lesser concentrations may still be present in the developing bud and bud scales. The relatively large volume of leaf-bud material which may be consumed by tits, may contain sufficient tannins to significantly reduce protein availability. Tannins present in the gut of caterpillars may also affect protein availability later in the season.

Secondly, the calcium intake of laying birds increases dramatically. Virtually the whole of the calcium required for eggshell formation must be ingested in the day preceding the morning the egg is laid. The calcium obtained from common dietary arthropods, such as spiders or caterpillars, is insufficient to meet the requirements for eggshell formation and tits must forage specifically for calcium-rich items such as snail shells (*Chapter 5*). Constraints relating to the time involved in foraging for sufficient calcium-rich items have been discussed earlier, but there are also possible physiological considerations.

Calcium concentrations in snail shell are 300 - 400 × higher than in normal arthropod prey (Graveland & Van Gijzen 1994), and a minimum of around 124 mg dry weight of snail shell must be consumed each day (calculated from values in Graveland 1995). Consequently, the calcium ion concentration in the digesta may rise by at least one or two orders of magnitude, which one would expect to have major implications for the pH of the crop and stomach. If crop and stomach pH rises, the efficiency of digestion of protein will be dramatically reduced.

Most calcium consumption by tits and other species where it has been examined, such as goldcrest (*Regulus regulus*), firecrest (*R. ignicapillus*) (Graveland 1995), red-cockaded woodpeckers (*Picoides borealis*, Repasky, Blue & Doerr 1981), and domestic hens (Gilbert 1983) occurs in the evening. This is believed to be largely a result of the timing of eggshell formation: in most species, eggshell formation takes place at night, presumably because the newly formed shell is fragile and limits the mobility of the female (Fogden & Fogden 1979; Schifferli 1979b). The evening consumption of calcium, however, may also serve to minimise interference with the digestion of food. Graveland (1997) thought this unlikely, since domestic fowl are able to maintain a high food intake and egg production on a diet of 2% calcium. However, as I have discussed above, extrapolations from poultry to tits should be used and treated with caution. Further, different enzymes require different pHs for optimum activity: pepsin, the primary gastric enzyme for protein digestion, requires very low pH. In contrast, enzymes present later in the alimentary tract, operate in more alkaline conditions. The ingestion

of calcium may, therefore, disproportionately and detrimentally affect protein digestion. This may also provide an additional explanation for the temporal distribution of nectar foraging discussed earlier.

Finally, the influence of food quality on egg production in great tits was recently examined by Nager *et al* (1997). In a diet supplementation experiment similar to that described in *Chapter 3*, birds were provided with either sunflower seeds (high energy but low quantity and quality of protein) or mealworms (high energy and high quantity and quality of protein). The provision of supplementary food resulted in an advancement of mean laying date and a slight increase in mean clutch size. However, there was no significant difference in elicited response between supplementary diets. Nager *et al* concluded that there was no evidence for any protein constraint on egg production. How can one explain the discrepancy between the findings of Nager *et al* and the present study?

Differences between studies in the nutritional composition of food supplements used may, perhaps, partially explain the observed discrepancy: in particular, sunflower seeds contain approx. 27% protein (although it is of low quality) compared with 0% protein in the animal fat supplement used in *Chapter 3*. I believe it unlikely, however, that this is the sole explanation for the difference in results. From Nager *et al*'s description, the woodland in which their study was conducted was more mixed, in terms of tree species, than my own oak-dominated study site. As discussed earlier, the increased presence of non-oak species may result in a greater availability of arthropods (a source of high quality protein) earlier in the breeding season. The discrepancy in findings, however, may also indicate an important difference in the constraints on egg production experienced by great tits and blue tits. Early in the breeding season, great tits have a largely different foraging niche to that of blue tits (Hartley 1953; Gibb 1954; Perrins 1979; Woodburn 1997); in particular, a significant proportion of foraging is performed on the ground. If arthropods were more abundant in ground litter than in the canopy at this time (particularly in oak-dominated woods), then the diet of early breeding great tits may be significantly more insectivorous than blue tits breeding in the same habitat, and energy supply would probably be expected to limit egg production first. Support for this hypothesis comes from Betts (1955) study of Parid gizzard contents, where great tits were found to contain a dramatically lower proportion of oak bud tissue than blue tits.

## Conclusions

Simplistic balance calculations of essential amino acid and calorific content in arthropods compared to estimated requirements for laying, suggested that the intake level needed to satisfy energy requirements was about four times greater than that which would lead to amino acid limitation. However, such calculations may be a significant misrepresentation of the nutritional scenario experienced by laying blue tits, particularly those breeding early in oak-dominated woodland. Arthropods may be extremely scarce and the diet may contain a high proportion of low protein quality plant tissue. Foraging at high energy content plant secretions may be favoured in order to satisfy daily energy requirements and allow selective foraging for scarce EAAs and calcium. Further, information regarding efficiency of digestion and absorption of different foods are lacking and there are good reasons to suspect major differences and changes in utilisation efficiencies. Consequently, it becomes increasingly possible that EAA availability may constrain egg production. Experimental provisioning with supplementary food of differing protein quality would appear to confirm that such nutritional constraints exist.

Finally, even within Parids, nutritional constraints on egg production may vary as a result of differences in habitat type or species specific foraging niche. Consequently, these factors must be taken into account when making comparisons or generalisations between studies.

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# Appendix 1

## Nestling diet and the amino acid composition of invertebrates

A systematic change in the composition of the diet fed to chicks has often been recorded in blue tits and great tits. Although caterpillars form the majority of the chicks' diet throughout the rearing period (Betts 1955; Minot 1981; Torok 1986; Cowie & Hinsley 1988) a number of researchers have found that the proportion of spiders in the diet increases steadily after hatching, reaches a peak around day 5 or 6, and then gradually declines (Royama 1970; Betts 1955; Tinbergen 1960; Van Balen 1973; Cowie & Hinsley 1988; Woodburn, thesis). Further, this pattern occurs irrespective of habitat, time of season or fluctuations in natural spider abundance. Royama (1970) interpreted his findings as implying that "spiders have some special nutritional value, important for the growth of nestlings at an early stage, which is not found in other types of food". He did not, however, offer any suggestion as to what specific nutrient(s) they might provide. In 1993, Gosler reported, anecdotally, that spider proteins were rich in the sulphur-containing amino acid cysteine. Feather keratin is also rich in this amino acid and Gosler proposed that the pattern of spider provisioning may be to provide the young with adequate supplies of cysteine during the period of maximal feather development.

From Figure 2.8b, we can see that spider protein has only slightly higher levels of cysteine than caterpillars. However, as a result of greater total protein content, cysteine and methionine levels are about 50% higher in spiders than caterpillars, in terms of SAA per unit dry mass. It is debatable, though, whether such a difference would be sufficient to drive the dramatic diet selection seen during chick rearing. At the time of chick rearing, spiders are considerably less abundant than caterpillars. Therefore, the increased SAA concentration in spiders could quite possibly be outweighed by the increased time spent obtaining them (time that could be spent providing the young with caterpillars). Spiders, however, also have vastly higher levels of the sulphur-containing amino acid taurine which, as discussed earlier, can have a sparing effect on an animal's cysteine/methionine requirements. Thus, although spiders have only about 50% more cysteine, on average, than caterpillars, their high taurine content would probably be sufficient to satisfy the chicks' requirements for bile formation, freeing up all the cysteine in the diet for other uses.

In his proposal, Gosler (1993) states that feather development in the great tit begins at around day 5 and that feather growth rate is maximal between day 5 and day 9 - when the quills rupture. However, in agreement with Woodburn (1997), I would argue that from Gosler's own graph of chick development and from personal experience, the greatest period of feather

development occurs in the second half of the nestling period. Indeed, I would interpret Gosler's graph as showing that rapid feather development (assumed to be the period of maximum cysteine requirement) does not begin until around day 8. By this stage, spiders make up only a small and still decreasing proportion of the nestlings diet. Therefore, is it possible that, early in the rearing period, tit nestlings actually require the dietary taurine itself, and not just for its sparing effect on cysteine?

Most research on the dietary requirement for taurine has been carried out on mammals; however, there is considerable evidence that many post natal mammals are unable to synthesise taurine at a sufficient rate; largely as a result of incompletely developed enzyme systems (review - Hayes 1976). As a result, taurine is generally present in high levels in maternal milk, especially in the colostrum; the milk produced early in the post natal rearing period (Erbersdobler *et al.* 1984; Gaull, Herminia & Wright 1985). Taurine is essential for bile formation and also has a number of extremely important functions in post-natal developing young. There is evidence that taurine is essential for the proper development and function of the central nervous system (Agrawal, Davidson & Kaczmarek 1971; Chanda & Himwich 1970; Sturman & Guall 1975; Rassin, Sturman & Gaull 1977 and refs. contained therin), and for the formation and development of the retina (Cohen, McDaniel & Orr 1973; Neuringer, Sturman, Wen & Wisniewski 1985; Sturman 1988). There is also some evidence from poultry that taurine is a growth factor, intimately associated with bone growth (Martin & Patrick 1961).

Thus, at least in mammals, taurine appears to be a fairly essential nutrient in the early development of young. Unfortunately, there is little information available regarding taurine requirements and synthesis in birds, and almost all of what there is comes from domestic poultry. There is some evidence that poultry chicks can synthesise taurine from sulphur-amino acid precursors (Hill & Martin 1973). However, since poultry chicks' 'natural' diet is largely plant derived (plants are generally devoid of taurine; Rana & Sanders 1986) we would perhaps expect the young of such a species to have evolved the capacity to synthesise taurine at an early stage of development. Further, the chicks are precocial and, therefore, hatch with well developed nervous systems and eyes. If they were unable to synthesise taurine from methionine or cysteine, the egg would have to contain all the taurine required for this development. Since taurine is not used in protein synthesis, and exists only as a free amino acid (Hayes 1976), it would perhaps be difficult to store enough taurine in the egg without causing problems, osmotic or otherwise. In altricial species such as Parids, these restrictions do not apply and, therefore, the enzyme systems may develop more slowly with the result that young nestlings require a dietary supply of taurine.

# Appendix 2

**Calculation of expected distribution of nestbox usage between 1992 and 1996 if blue tits chose nestboxes at random.**

## Yr1 - 1992

194 nestboxes: 43 occupied, 151 unoccupied

**Table A6.1**: Situation at end of Year 1

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 151 | 77.835 |
| Once | 43 | 22.165 |
| Twice | 0 | 0 |
| Three times | 0 | 0 |
| Four times | 0 | 0 |
| Five times | 0 | 0 |

## Yr2 - 1993

194 nestboxes: 57 occupied, 137 unoccupied

| | | |
|---|---|---|
| | 77.835% * 137 | = 106.634 boxes occupied **never** |
| & | 77.835% * 57 | = 44.366 boxes occupied **once** |
| & | 22.165% * 137 | = 30.366 boxes occupied **once** |
| & | 22.165% * 57 | = 12.634 boxes occupied **twice** |

**Table A6.2**: Situation at end of Year 2

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 106.634 | 54.966 |
| Once | 74.732 | 38.522 |
| Twice | 12.634 | 6.512 |
| Three times | 0 | 0 |
| Four times | 0 | 0 |
| Five times | 0 | 0 |

At the beginning of 1994, 76 additional nestboxes were deployed.

**Table A6.3**: Situation at beginning of Year 3

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 182.634 | 67.642 |
| Once | 74.732 | 27.679 |
| Twice | 12.634 | 4.679 |
| Three times | 0 | 0 |
| Four times | 0 | 0 |
| Five times | 0 | 0 |

## Yr 3 - 1994

270 nestboxes: 68 occupied, 202 unoccupied

| | | | |
|---|---|---|---|
| | 67.642% * 202 | = 136.637 | boxes occupied **never** |
| & | 67.642% * 68 | = 45.997 | boxes occupied **once** |
| & | 27.679% * 202 | = 55.912 | boxes occupied **once** |
| & | 27.679% * 68 | = 18.822 | boxes occupied **twice** |
| & | 4.679% * 202 | = 9.452 | boxes occupied **twice** |
| & | 4.679% * 68 | = 3.182 | boxes occupied **3 times** |

**Table A6.4**: Situation at end of Year 3

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 136.637 | 50.606 |
| Once | 101.909 | 37.744 |
| Twice | 28.274 | 10.472 |
| Three times | 3.182 | 1.179 |
| Four times | 0 | 0 |
| Five times | 0 | 0 |

## Yr 4 - 1995

270 nestboxes: 59 occupied, 211 unoccupied

| | | | |
|---|---|---|---|
| | 50.606% * 211 | = 106.779 | boxes occupied **never** |
| & | 50.606% * 59 | = 29.858 | boxes occupied **once** |
| & | 37.744% * 211 | = 79.640 | boxes occupied **once** |
| & | 37.744% * 59 | = 22.269 | boxes occupied **twice** |
| & | 10.472% * 211 | = 22.096 | boxes occupied **twice** |
| & | 10.472% * 59 | = 6.178 | boxes occupied **3 times** |
| & | 1.179% * 211 | = 2.488 | boxes occupied **3 times** |
| & | 1.179% * 59 | = 0.696 | boxes occupied **4 times** |

**Table A6.5**: Situation at end of Yr 4

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 106.779 | 39.548 |
| Once | 109.498 | 40.555 |
| Twice | 44.365 | 16.431 |
| Three times | 8.666 | 3.210 |
| Four times | 0.696 | 0.258 |
| Five times | 0 | 0 |

## Yr 5 - 1996

270 nestboxes: 62 occupied, 208 unoccupied

| | | |
|---|---|---|
| | 39.548% * 208 | = 82.260 boxes occupied **never** |
| & | 39.548% * 62 | = 24.520 boxes occupied **once** |
| & | 40.555% * 208 | = 84.354 boxes occupied **once** |
| & | 40.555% * 62 | = 25.144 boxes occupied **twice** |
| & | 16.431% * 208 | = 34.176 boxes occupied **twice** |
| & | 16.431% * 62 | = 10.187 boxes occupied **3 times** |
| & | 3.210% * 208 | = 6.677 boxes occupied **3 times** |
| & | 3.210% * 62 | = 1.990 boxes occupied **4 times** |
| & | 0.258% * 208 | = 0.537 boxes occupied **4 times** |
| & | 0.258% * 62 | = 0.160 boxes occupied **5 times** |

Situation at end of Yr 5

| No. of times occupied | No. of boxes | % Occurence |
|---|---|---|
| Never | 82.260 | 30.467 |
| Once | 108.874 | 40.324 |
| Twice | 59.320 | 21.970 |
| Three times | 16.864 | 6.246 |
| Four times | 2.527 | 0.936 |
| Five times | 0.160 | 0.059 |